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BENIGN PROSTATIC HYPERPLASIA

NIAMDD WORKSHOP PROCEEDINGS
February 20–21, 1975

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BENIGN PROSTATIC HYPERPLASIA

A Workshop Sponsored by the
Kidney Disease and Urology Program
of the National Institute of Arthritis,
Metabolism and Digestive Diseases

National Institutes of Health
Bethesda, Maryland
February 20–21, 1975

John T. Grayhack, M.D.
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Editors

DHEW Publication No. (NIH) 76–1113

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Stock Number 017-045-00069-5

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PREFACE

NANCY BOUCOT CUMMINGS

Recognizing that over ten per cent of admissions to general hospitals are for urologic problems, the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD) has had a major interest in initiating new research on diseases of kidney and urinary tract disorders. A study, Evaluation of Research Needs in Nephrology and Urology, is underway to identify "gaps" in research and to identify possible approaches to finding ways to prevent and arrest disease processes afflicting the population.

NIAMDD Advisory Council member, Dr. William L. Valk of the University of Kansas School of Medicine, indicated that benign prostatic hyperplasia was a disorder justifying high priority for new research efforts. Its incidence and prevalence in the male population over 45 years of age is significant, since signs and symptoms increase from an occurrence of about 45 per cent in 45 year old men to 80–90 per cent in 80 year old men.

Aware of the need for innovative research and of the value of interdisciplinary collaboration and interchange, the NIAMDD made the decision to sponsor a workshop that would bring together investigators from many disciplines: endocrinology, pathology, biochemistry, epidemiology, embryology, and anatomy, along with the urologists who over the years have maintained the primary concern for benign prostatic hyperplasia. The hope was held that resulting cross-fertilization of ideas might spur new approaches and cooperative efforts into basic and clinical research on the prostate gland.

The high quality of the program is attributable to the efforts of the planning committee, represented by the expertise of co-chairmen, John T. Grayhack, M.D. and Jean D. Wilson, M.D. and NIAMDD's M. J. Scherbenske, Ph.D. We are highly indebted to Dr. Oscar Hechter for the thought and effort he put into his overview of the conference, "Reflections on the NIH Workshop on BPH", which incisively focuses on the current state of research and of the potential areas for study. Special recognition is due to Ms. Carol A. Stovall, Grants Assistant, Kidney Disease and Urology Program, NIAMDD, who worked for the committee on arrangements and transcription of the tapes of the meeting.

We would like to express our appreciation to all the conference participants who contributed to a scientifically valuable meeting.

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INTRODUCTORY REMARKS

G. Donald Whedon

As the Institute of the National Institutes of Health with principal responsibility for the kidney and urinary tract, the NIAMDD is becoming more and more concerned with urological problems, not only in relation to the support of research but also the design of appropriate training for future urological investigators. In regard to benign prostatic hypertrophy, we are faced with a formidable problem in that the majority of men over the age of 60 have symptomatic enlargement of the prostate, and the combined costs of this disorder—social and economic—are incalculable. It is thus a real pleasure for me to inaugurate this workshop. It was conceived with several thoughts in mind, namely to review the current state of the problem and to bring together investigators from a variety of scientific disciplines to focus new attention on the disorder. A specific problem in stimulating investigation in an underdeveloped field such as urological research is whether we should induce those already in the field to seek broader experience in other disciplines or whether it is better to attempt to attract people from other disciplines into urological research. There is no clear-cut answer to this problem, and in this workshop we have combined both approaches in the hopes that as the result of cross pollination, urologists will be challenged to apply newer approaches to their old problems and non-urologists will be equally challenged to confront important problems outside their ordinary frame of reference.

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INTRODUCTORY REMARKS

JOHN T. GRAYHACK

The stimulus for the meeting, as Dr. Whedon said, was the increasing amount of information from individuals and groups with different backgrounds concerning numerous aspects of prostatic hypertrophy and a similar expansion of information concerning normal prostate growth and the normal endocrine profile of the human male. We hope that by assembling here we can exchange information and catalyze the exchange of ideas and information. We also hope that the published report of these proceedings will be a useful summary of current pertinent knowledge of BPH and will serve as a useful starting point for investigators who develop an interest in this common problem of man.

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INTRODUCTION

JEAN D. WILSON

The organization of this meeting was based upon three underlying assumptions: First, the growth of the prostate, normal and abnormal, is mediated by testicular hormones, and benign prostatic hypertrophy does not occur in castrated men. Although this assumption has not been proven, the evidence in its favor is formidable. It does not imply, however, that endocrine abnormalities are necessarily primary in the etiology, only that at a minimum a permissive role for testicular hormones is essential for its development. Second, there is a remarkable species specificity in prostatic hypertrophy in that the disorder is universal only in the aging man and dog. Third, considering its importance as a medical problem in virtually all aging men, the disorder has attracted little attention from medical investigators. Consequently, it was hoped that the bringing together of scientists from different disciplines to focus attention on this underdeveloped area might provide a significant stimulus to the elucidation of its pathophysiology.

Indeed, even if we assume that the first two assumptions are correct it is not clear how future studies should be designed. For example, it is uncertain whether the species specificity of prostatic hypertrophy is a function of the endocrine system, i.e. whether qualitative or quantitative differences exist in the circulating hormones, or whether the species specificity is located in some unique receptor system in the prostate itself. In other words, it is not known whether the species specificity is due to some unique feature of the male hormones or is the result of some peculiar feature of the target tissue. Second, even if the nature of the species specificity were resolved, it would still be uncertain whether prostatic hypertrophy is in fact a phenomenon of aging. As demonstrated by Swyer, the male prostate increases in size during adolescence, reaches a plateau at about age 20, and remains constant in size until about the age of 50 when it begins to increase again (1). This 20–30 year lag phase in the growth of the prostate has been well documented in many studies, and it has been assumed generally that whatever the etiology of prostatic hypertrophy, it must be a disorder of aging. However, Swyer's data may have been overinterpreted. On the basis of unpublished pathological studies in our laboratory in which all prostates of accident victims of all ages were examined, characteristic early histological features of human prostatic hyperplasia could be identified in the periurethral region of the gland in some men in the 20–30 year age range. If, in fact, the disorder commences in the 20's, it is a mistake to focus only on the end stage when the gland has become enlarged in size.

Consequently, since it is not certain whether in the strictest sense prostatic hyperplasia is a disease of aging, we have attempted to assemble a group that is broad enough to encompass a variety of possibilities. On the one hand, some unique event may occur in the middle-age man either in the endocrine system or

1.) Swyer, G. J. M. Postnatal growth changes in human prostate. J. Anat. (Lond.) 78:130, 1944.

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in the prostate that results in a second burst in prostatic growth with its well known pathological consequences. On the other hand, the disorder may be the end stage of events that commence in late adolescence or early maturity and eventuate in prostatic enlargement only in the terminal phases of the process. Thus, attention will be focused on the total overall pattern of endocrinology in the male and on the physiology as well as the pathology of prostatic growth. We hope, specifically, that definable questions can be elucidated at this meeting and that this effort will serve as a stimulus for young investigators in the field.

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DEVELOPMENTAL AND COMPARATIVE ANATOMY OF THE PROSTATE

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Introduction

The pelvic urethra with adjacent mesenchyme, from which the embryonic prostate develops, is heterogeneous in composition (1,2). Its upper half, though basically derived from the endodermal urogenital sinus, has posteriorly a component of mesonephric mesoderm extending down from the bladder. In later development, the mesenchyme in this area becomes smooth muscle continuous with that of the bladder. The lower half of the pelvic urethra develops entirely from urogenital sinus. It receives most of the prostatic ducts posteriorly, and its mesenchyme anteriorly differentiates in part into striated muscle. At the junction of these two areas, a third component, the Wolffian duct, forms a small island of mesodermal tissue interposed into the posterior wall. At the ninth week of development, prior to budding of the prostatic ducts, these three areas are continuous, with no sharp demarcations or histologic differences between the different epithelia and stromas (Fig. 1).

This presentation will review the evidence on the subsequent development of the prostatic duct system with reference to four aspects: 1) the relative contribution of the three different areas to the composition of the prostate; 2) the nature of local epithelial stromal interactions in prostatic differentiation; 3) similarities in development between different species; 4) the relevence of embryonic development for understanding adult pathology.

FIGURE 1. Sagittal view of prostate in 9 to 10 week embryo. Wolffian duct joins midpoint of pelvic urethra. Bladder and penile urethra are not seen. Rectum is at left. Original magnification, 11x.

Composition of the Embryonic Prostate

Androgenic hormone is the embryonic inducer which triggers the budding of ducts from the urethral lining into the adjacent stroma (3,4). Duct development begins at about the twelfth week, shortly after the embryonic testes begin to produce

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and secrete testosterone. Duct budding is not limited to a short segment but occurs more or less continuously from the bladder neck to the origin of the penile urethra, most buds originating along two rows at the lateral angles of the posterior urethral wall (2,5).

The subsequent course of duct development in the prostatic mesenchyme has been traced in detail by Lowsley (5). He defined a *posterior lobe* originating from the two rows of ducts in that segment of the urethra below the verumontanum. The two *lateral lobes* arose along the urethral segment just lateral to the verumontanum. Those ducts which budded above the veru were noted not to undergo complete development and were therefore not regarded as truly part of the prostate. There were two other prostatic lobes which were not part of the postero-lateral rows of ducts. The *middle lobe* developed from a small cluster of ducts on the crest of the veru just above the ejaculatory duct openings and therefore in the area between the lateral lobe duct orifices. An *anterior lobe* was described, derived from ducts budding off the anterior urethral wall. The anterior lobe has not been given further consideration since it regularly atrophies by the time of birth.

Lowsley felt that the posterior lobe was embryologically different from the other lobes. Generally speaking, in constructing models of organogenesis, it is important to substantiate apparent developmental differences by additional evidence gained through adult morphology, biochemistry, or other techniques. It was accordingly proposed that the exclusive development of carcinoma in this lobe was a demonstration of that difference in the adult. However, the concept that carcinoma is restricted to the posterior lobe has since been proven incorrect (6). Furthermore, Lowsley never indicated that there were any histologic differences between lobes, and after birth there are no boundaries visible between them. Since no other evidence from adult anatomy or any other source has ever confirmed any differences between any of Lowsley's lobes, it is doubtful whether they have any developmental or adult significance.

However, there exists in the adult prostate a morphologic heterogeneity of probable developmental significance (7,8). It was apparently overlooked by Lowsley because of minor inaccuracies in his observations, and because his conclusions from embryos were not checked against adult morphology. The cluster of duct buds on the verumontanum, which Lowsley equated with the middle lobe, does not arise independently of the ejaculatory ducts. The ducts are oriented to form a small circle completely surrounding the junction of the Wolffian ducts with the urethral mucosa. The course of this group of duct buds continues to follow that of the Wolffian ducts superiorly through the prostate, surrounding the ejaculatory ducts as a rosette (Fig. 2). As these ducts branch, they fan out mainly laterally but continue to enclose the ejaculatory ducts by branching both anteriorly and posteriorly to them.

In the adult, the stroma of these ducts is compact and blends with the similarly compact stroma of the ejaculatory ducts. The remainder of the prostate has a sharply contrasting, loosely woven stromal texture (Fig. 3). Unlike the remainder of the prostate, glands developing around the ejaculatory ducts show an elaborate pattern of epithelial folds into gland lumina. This mucosal pattern is also seen in the seminal vesicles and ejaculatory ducts. Characteristics of the individual epithelial cells also suggest that these three structures are more akin to each other than to the remainder of the prostate. In the newborn infant, squamous metaplasia is much more common and more fully developed in this zone around the Wolffian ducts than elsewhere in the gland.

Thus, postnatal morphologic observations supplement embryonic histology and strongly suggest that one group of prostatic glands may be of Wolffian duct derivation rather than originating from the urogenital sinus. This proposed duality requires still further confirmation by additional techniques. It has recently been found, for example, that the

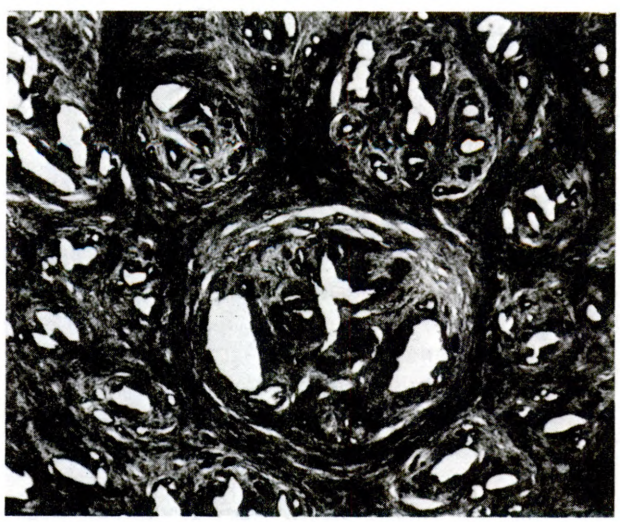

FIGURE 2. Rosette of prostatic ducts surrounding ejaculatory duct above verumontanum in newborn infant. Original magnification, 5x.

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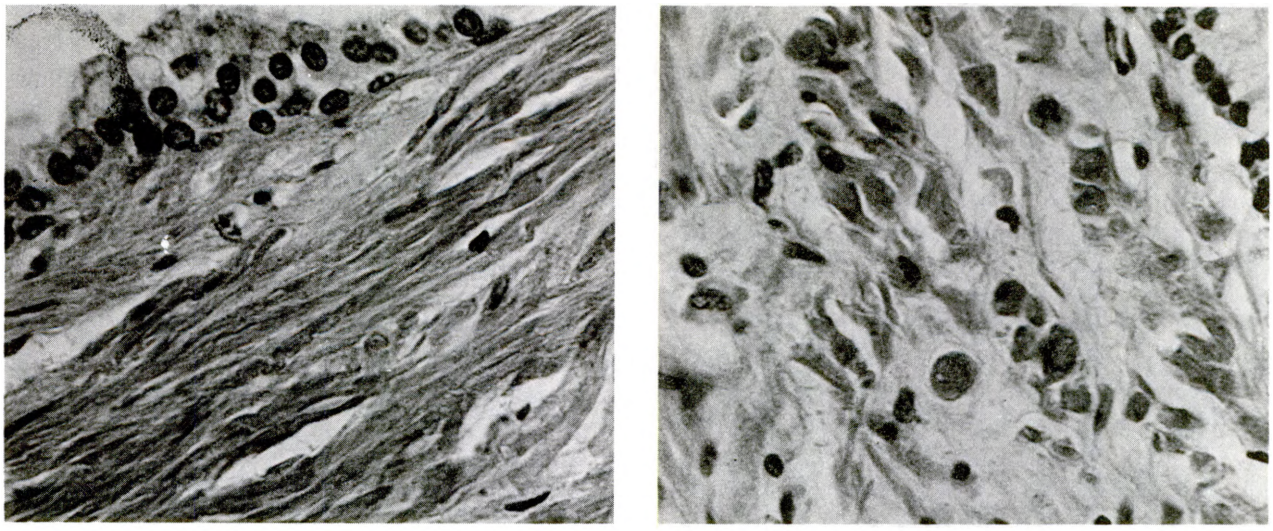

FIGURE 3. Differences in stroma between compact muscle of central zone (left) and loose muscle of peripheral zone (right). Original magnification, 180x.

Wolffian duct develops under the inducing stimulus of testosterone, while the hormone which triggers urogenital sinus differentiation in the male is dihydrotestosterone (3,4,9). It would be important to establish similar biochemical distinctions in these different regions of the prostate, both in embryos and after birth.

In the fully developed human prostate, the two glandular components are contiguous and form a disc of tissue flattened in the antero-posterior dimension (2,7) (Fig. 4). Those ducts of proposed Wolffian duct origin form the superior disc sector—about 25% of its circumference—and radiate upward from the verumontanum, which lies at the disc center. This sector has been called the "central zone" because of its relationship to the axis of the Wolffian duct system. The posterior urethral wall is embedded in the disc from its center downward. The ducts of the remainder of the prostate ("peripheral zone") bud laterally from this segment. Above the verumontanum, no prostatic ducts originate (2,5). This urethral segment angles sharply forward away from the prostatic disc to accommodate a sleeve of smooth muscle fibers which is continuous with the bladder neck above. This sleeve is a sphincter which functions in urinary continence and which represents the differentiation end-point for the mesenchyme of the upper half of the pelvic urethra.

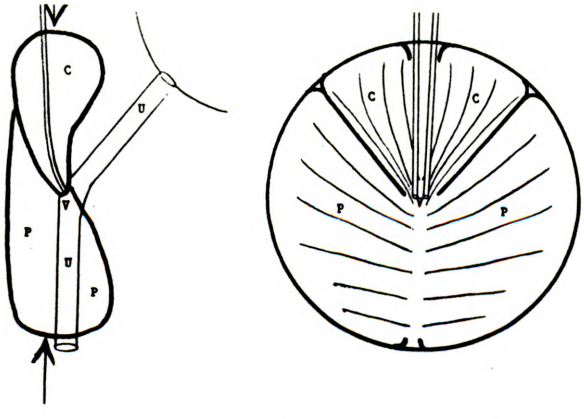

FIGURE 4. Diagram of relationship between prostate zones, ducts, and urethra. Sagittal view and coronal section along course of ejaculatory ducts. C=central zone, P=peripheral zone, V=verumontanum, U=urethra.

Epithelial-Stromal Interactions

The urethral buds which form in this upper urethral segment do not complete development. They remain as vestigial structures—the periurethral glands—which branch to a limited extent near the urethral mucosa or end blindly in the inner layers of the sphincteric muscle. A reasonable explanation for their lack of development is that this mesenchyme of urinary excretory differentiation is not a competent stroma to induce glandular development in epithelium (2). Organ specific stromas have a powerful influence on epithelial differentiation, extending even into adult life. If embryonic urethral epithelium is mixed in tissue culture with embryonic stroma from either urogenital sinus or

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Wolffian duct, it will develop branching prostatic glands, but if mixed with a non-genital mesenchyme, no gland formation occurs (10,11).

However, in order to respond to stromal induction, the epithelium itself apparently requires an initial priming influence. There is evidence that the responsiveness of the urethral epithelium is acquired through a direct inductive effect of testosterone on the embryonic epithelial cells. Embryonic urethral epithelium in rats which has been protected from the early embryonic wave of testosterone stimulation will not respond with gland production to an inducing prostatic stroma. Responsiveness can be restored by a brief exposure to exogenous androgen (12).

The morphology of the adult male urethra suggests that this androgen-induced epithelial priming has occured diffusely and not just in areas where the prostatic buds arise. In the male, the entire transitional epithelium from the bladder neck to the origin of the penile urethra has been uniquely modified. As its basal cells mature toward the surface, they never develop the abundant cytoplasm and flattened, squamous appearance which is seen in the bladder and along the female urethra. Instead, the cells remain narrow and small, and their surface layer becomes a tall, columnar, glandular epithelium. From the glandular surface, tiny intramucosal acini frequently bud downward and are contained entirely within the epithelial thickness (Fig. 5). This appearance is seen in infancy and childhood and hence does not require persistant androgen stimulation for its maintanance. If morphology truly reflects embryonic determination, then the upper segment of the urethra has a similar epithelium and differs from the lower segment only in the direction of its stromal differentiation. The epithelium is presumably responsive to gland development but lacks appropriate inductive stroma.

Post-Natal Development

Organogenesis in many systems is not restricted to the fetal period but may continue post-natally. Certainly this is evident in the prostate, where an essentially embryonic morphologic appearance persists in the entire gland until puberty, when differentiation is finally completed. However, there are still other aspects in which evolution of structure continues well into adult life and merges into the pathology of later years.

The distinctive epithelium lining the prostatic urethra shows progressive elaboration of its embryonic glandular potential throughout adult life (McNeal, to be published). There is a wide spread variation between individuals, but comparison of urethral morphology in successive decades shows greater and greater frequency of intramucosal acini and even of short ducts that extend through the mucosa and branch into the stroma (Fig. 6). These changes are seen mainly above the verumontanum, where in many men they produce an increased population of periurethral glands in later life.

It is in the periurethral glands, exclusively above the verumontanum in the area of the urinary sphincteric stroma, where BPH arises in later adult

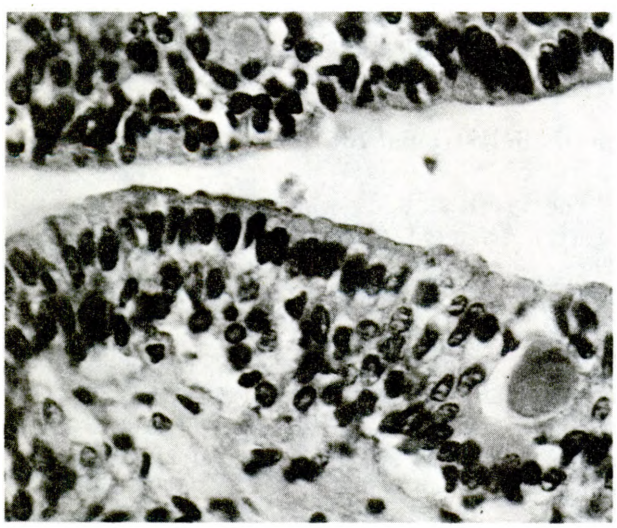

FIGURE 5. Urethral mucosa above veru in young adult showing columnar surface and mucosal acini. Original magnification, 180x.

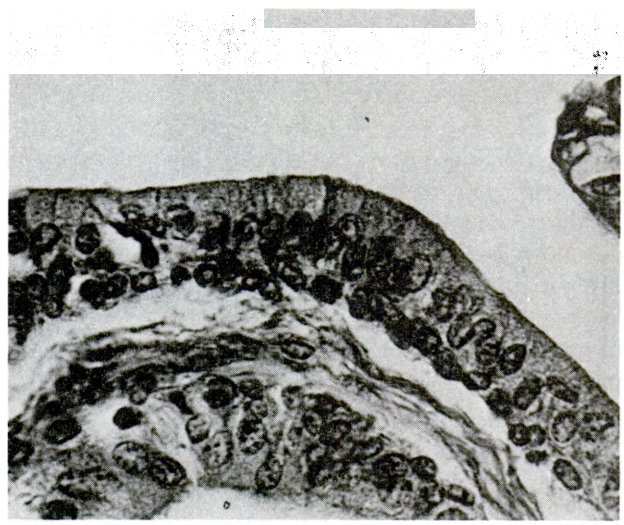

FIGURE 6. Urethral mucosa in 46 year old man showing striking glandular evolution and periurethral duct buds. Original magnification, 180x.

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life (2). The morphology of this condition is strongly suggestive of distorted inductive interactions (2,13), but it is not clear whether the epithelium or stroma is the component at fault (4). It is also not clear whether the periurethral glands involved are those present at birth or those which arise later.

In the central zone of the prostate, as in Wolffian duct derivatives generally, no continuation of structural evolution is seen in adult life. Hyperplasias, tumors, and carcinoma are correspondingly uncommon. In the peripheral zone of the prostate, however, new budding of glandular structures is often seen in men past 50 (8). Such creation of new architecture always requires stromal interaction and may depend on mechanisms similar to those of initial embryonic organogenesis. In this case, in contrast to BPH, the stromal component is not prominent, and there is not a distortion of pattern, so no nodules are produced. Normal structure is, therefore, well imitated except for progressively closer packing of glands. The process is significant in that its end result is often an evolution into carcinoma.

Homologies

In the baboon and monkey, there are two separate divisions of the prostate, which unlike the human gland are externally visible (15,16). One of these originates from ducts which surround the ejaculatory ducts and parallel their course to the verumontanum (17). Hence this "cranial lobe" may be homologous to the central zone in the human and may be of Wolffian duct origin. In the rat, the coagulating gland has some morphologic similarity to this division of the simian prostate (17) and to the human central zone. The coagulating gland is said to develop inside the capsule of the seminal vesicle (10), and these two structures have similar features of estrogen and cortisone responsiveness, which are different from the remainder of the prostate (18). On morphologic grounds, Van Wagenen proposed that a coagulating gland should be searched for in humans in the prostatic ducts immediately surrounding the ejaculatory ducts (17). In these observations, there is further evidence for the Wolffian duct origin of a portion of the prostate and for the persistence of this design in evolution.

In the canine prostate, no ducts are seen in a rosette around the ejaculatory ducts, and there is no tissue in the area occupied by the human central zone (McNeal, to be published). The seminal vesicles are also missing. This suggests that in this species, the Wolffian duct stromal territory has failed to induce any secondary structures. Hence, the only tissue present is that representing the human peripheral zone. It is possible, however, that a combination of the functions of central zone and seminal vesicle into this area may have altered this tissue so that it is no longer strictly homologous to pure peripheral zone tissue.

In the dog, the urethral lining shows much less glandular differentiation than the human, and above the verumontanum, it is consistently absent. The epithelium there is nearly identical to the bladder. There are no intramucosal acini and no budding of periurethral glands. The dog seems to lack entirely the human equivalent of periurethral glands of urethral mucosal origin. Abundant glandular tissue is seen near the urethra, but it seems always to represent early branches of the main prostatic ducts on their way to the periphery.

Postnatal, progressive alteration in gland structure often leads to benign hyperplasia in the canine prostate (19). However, this condition is morphologically different from the superficially similar BPH of humans (15,19,20) though the dog is often cited as a model for BPH. Canine hyperplasia shows mainly acinar enlargement without budding or generation of new architecture. Hence, it more resembles the direct effect of androgen administration than the embryonic epithelial-stromal interactions of man. The canine process is diffuse rather than nodular. It does not involve the periurethral glands, since these are not present, nor any other vestigial structures. It derives instead from the original prostatic tissue and does not involve the urinary sphincter.

Discussion

Adult structure, embryonic morphology, and comparative anatomy form interlocking bodies of evidence about human organogenesis. Probably the use of all three is necessary to construct a coherent model of developmental morphology. However, morphology by itself can only develop hypotheses and can never yield a full understanding of developmental dynamics. As a result of more sophisticated techniques, the gap in morphologic models of prostate embryogenesis are now being filled. It is now clear that inductive interactions between organ-specific stroma and urethral epithelium are necessary for formation of the prostate, and it is established that testosterone is the trigger whose production from the fetal testis times the onset of these inductions.

Further studies are needed to determine what

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role embryonic processes may have in adult pathology. There is some morphologic evidence to support the hypothesis that certain facets of embryonic development may continue into adult life. Furthermore, wherever a pathologic process involves generation of new glandular architecture in adulthood, as in BPH, the possibility of epithelial-stromal interactions of embryonic pattern must be considered. If this is a valid consideration, it should be remembered that three elements always play a part in embryogenesis-the reading of the DNA code within the individual cell, short range interactions between neighboring elements, and long range humoral stimuli. In the case of BPH, much is being learned about the last factor, but very little is yet known about the first two.

Confirmation by more sophisticated techniques is also needed for the morphologic concepts of similarities and differences between species. Only if these similarities and differences turn out to be variations on a single theme, traceable through evolution, can data be properly extrapolated from other animals to man. In the case of the dog, considerable morphologic evidence suggests that several aspects of embryogenesis differ from the events in man. Directly relating to that fact, the events in the pathogenesis of canine prostatic hyperplasia appear to differ from those leading to human BPH. In particular, epithelial-stromal interactions may be involved in BPH but appear absent in canine hyperplasia. Therefore the use of the dog as a model for human prostatic disease should depend on support by other additional evidence that sufficient comparability exists despite morphologic findings to the contrary.

Summary

Prostatic development is characterized by diversity in the character of tissue interactions at different levels of the prostatic urethra. Most prostatic ducts develop in the lower half of the pelvic urethra from tissue of urogenital sinus origin. About 25% of the glandular tissue develops at the midpoint of the urethra, immediately around the ejaculatory ducts and has a strikingly different morphology. This region of the prostate may be of Wolffian duct origin and is probably homologous with the simian cranial lobe and the rat coagulating gland. Such accessory glandular structures of possible Wolffian duct origin are notably absent in the dog prostate.

Testosterone has been identified as the long-range humoral inducer which triggers developmental epithelial-stromal interactions in prostatic organogenesis. Short range inductive interactions are of comparable importance but are little understood. There is morphologic evidence that lack of development of duct buds above the verumontanum may result from deficient inductive stimulus by the local stroma. Instead of supporting prostatic development, the mesenchyme here differentiates into a urinary sphincter.

Above the verumontanum, continuing tissue changes after birth suggest persistent embryonic evolution. The urethral mucosa here continues a course of progressive glandular differentiation in adult life. The stroma and periurethral glands above the veru interact later in life in producing the nodules of BPH. In the dog, periurethral glands and urethral glandular differentiation are lacking. Hence canine prostate hyperplasia is probably not homologous with human BPH.

Acknowledgement: I would like to acknowledge the valuable assistance of Mrs. Eva Schick in the preparation of histologic material

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DISCUSSION

G. WILLIAMS-ASHMAN: Do I understand you correctly to say that you believe that the coagulating gland of the rat is derived from the Wolffian Duct?

J. E. MCNEAL: That was my postulate.

WILLIAMS-ASHMAN: What is the evidence? The coagulating gland has been called the anterior prostate since many a year.

MCNEAL: The coagulating gland of the rat?

WILLIAMS-ASHMAN: Yes. This structure certainly has some remarkable morphological and biochemical similarities to other lobes of the rat prostate.

MCNEAL: The work of VanWagenen, the work of Tisell, the similar estrogen responsiveness of the coagulating gland and the seminal vesicle, and the morphologic similarities between the human, monkey and rat coagulating gland are all compatible with such an interpretation. My feeling is that the role of a morphologist is not really to prove anything but to provide hypotheses on the basis of morphologic similarities. It is up to the biochemist to prove us right, or wrong. I think there is sufficient evidence for a Wolffian Duct origin of the coagulating gland that it ought to be investigated whether the central zone in the human, the cranial lobe in the monkey, and the coagulating gland in the rat are homologous and whether they have features indicating that they come from Wolffian Duct. I didn't mean to indicate that I thought I had proved that.

J. D. WILSON: Do you exclude the possibility that Mullerian Duct remnants may be contributing to any or all of these?

MCNEAL: No. I just hadn't even considered it at all. I see no reason why one has to discount it. It's certainly possible.

L. M. FRANKS: I wonder why it is that the embryologists haven't really studied this properly because this is a very important discussion. It is an important point that you raised, and people have discussed this for many years and all the references on the subject go back to Lowsley, which is really quite a long time ago. One wonders why the embryologists haven't approached this problem as carefully as they have done with other organs.

MCNEAL: Well, it occurs to me that there are two reasons. First it is the natural tendency to try to deal with disciplines separately. There's embryology over here, comparative anatomy over here, and adult morphology over here. I think it's only when you put them all together that you begin wondering about things that you might not think about if you deal with embryology alone. Second, the prostate after birth continues to look pretty embryonic, and it doesn't really become a fully differentiated gland until puberty. Some of the features that I demonstrated are not obvious just by looking at the embryo, but once seen in the adult, one has to go back and see where they came from as you suggested.

FRANKS: How common is that glandular metaplasia? I think these changes in the postatic urethra are things I must have seen but never recognized before. One just accepts it as a finding that one sometimes sees. I don't know how common it is.

MCNEAL: Well, I have to plead guilty of showing my best example, naturally. It occurs to a lesser degree and usually at an older age than this in many men. As you look at the urethras of newborn infants, a fair degree of variability already exists so that it becomes difficult to be sure in the individual

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case whether a urethra has always looked like this. Only when one examines them at the age 50 or 60 and finds changes that surpass anything you see in the infant, is it clear what has evolved. In the individual case like this, one would never find anything in an infant or child or young man that approached the metaplasia I showed you here.

WILSON: In defense of embryologists, I would like to say that the methodology involved in that particular organ is complex and that the particular difficulty is that the development of the male urogenital sinus is so asymmetrical that the Müllerian Duct has actually started to deteriorate before the differentiation of the urogenital sinus is complete. Thus, deterioration going on at one end and development at the other so that it is difficult to trace the origin of individual cell lines.

O. HECHTER: When you speak about a new architecture being created in adult BPH as against a hyperplasia that distorts an existing architecture, I think that is one of your key points.

MCNEAL: Yes, it is.

HECHTER: Will you elaborate?

MCNEAL: I think that this will probably fall more into Dr. Franks' province. Not only in the prostate, but in other organs as well, it's customary for pathologists to talk about hyperplasia in two different senses—architectural hyperplasia and epithelial hyperplasia with no change of architecture. In dealing with the breast, for example, we say, "this woman shows ductal hyperplasia of the breast". And, as you look, you don't see any more ducts than you saw before, but they're filled with epithelial cells. Now that's the sort of thing that has happened in the dog prostate. On the other hand, in other organs the term hyperplasia, as for example in the endometrium, is used to mean that preexisting glands are budding off little new daughter glands, which in turn are budding off little new glands from them so that a whole new branching system of ducts develops whereas before only one trunk duct was present. I believe the latter situation, branching of new ducts with development of a whole new architectural system of ducts, is occurring in BPH.

HECHTER: This does not involve all lobes of the prostate?

MCNEAL: It involves what I would prefer not to consider truly prostatic tissue at all. Going back again to Lowsley, it has been thought that the periurethral glands from the verumontanum up to the bladder neck are not part of the true prostate. And, it is in the periurethral glands, above the verumontanum and from there to the bladder neck that BPH does seem to develop.

I. LEAV: I was wondering if you have any comments on the squamous metaplasia that is seen in newborn infants usually involving the periurethral region selectively? Do you consider this to be a specific response to estrogens and do you think that this regional sensitivity plays any role in the development of BPH?

MCNEAL: Yes, I think it's generally accepted that the metaplasia is estrogen induced. I think it might be worth further investigation to see whether this is indeed the case. But the squamous metaplasia that I have seen in newborns mainly involves the ducts which are immediately around the ejaculatory ducts, which I have called the central zone, not the periurethral gland and not the bulk of the prostate that originates below the central zone.

I. LASNITZKI: I wondered how the presence of androgen receptors in the mesenchyme can be reconciled with Cunha's suggestion that it only plays a supporting role?

MCNEAL: As you indicate, in Dr. Cunha's work, it appears that the mesenchyme plays only a passive role if one places unstimulated, non-androgen exposed mesenchyme next to androgen exposed epithelium, the epithelium develops into ducts. But if you switch it around so that the epithelium hasn't been exposed and the stroma has, you don't get anything. This suggests that the stroma doesn't play a very positive role. However, in other studies he has combined this epithelium, androgen primed and ready to go, with a foreign stroma, and no buds develop. This may mean that something has already been predetermined both in prostate stroma and epithelium to undergo male genital tract differentiation. Now something needs to tell the prostate when to go, and that's the purpose of the testosterone. You don't need to tell both the stroma and the epithelium when, you only need to tell one, and then the program built into both begins to unfold itself. I think that's a possible interpretation, don't you, Dr. Lasnitzki?

LASNITZKI: Would you expect formation of prostatic ducts if untreated epithelium is combined with mesenchyme that had been previously exposed to testosterone?

MCNEAL: I would expect that.

LASNITZKI: The epithelium would become stratified instead?

MCNEAL: Right. Right. The hypothesis is that both epithelium and stroma are involved but that epithelium alone is responsive to the timing signal. The fact that plain androgen-treated stroma next to non-androgen treated epithelium does not result in

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differentiation doesn't mean that they are not both involved, but that the triggering signal is missing. I don't know how I can answer it better. This brings us back to the point that in any of these embryonic events there are probably four types of influence: the genetic code Dr. Franks is talking about, testosterone or another hormone, the epithelial-mesenchymal interaction, and the interaction between individual cells. But they are all acting at any point in time. The question is what role does each play at this particular point rather than which is doing the job to the exclusion of the others.

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SOME BIOCHEMICAL CHARACTERISTICS OF THE HUMAN PROSTATE IN RELATION TO ITS BENIGN HYPERPLASIA

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It could be said that much of our present knowledge of the prostate gland rests on seven pillars of observation and experiment:

(1) Prostate glands are unique attributes of mammals.

(2) The initial morphogenesis of prostate glands from the embryonic urogenital sinus requires androgenic steroids produced by the fetal testis.

(3) The growth and functional differentiation of the prostate at puberty, as well as the maintenance of normal prostatic size and secretory activity throughout the lifetime of the adult male, are critically dependent upon the levels of circulating androgens.

(4) The existence of distinct lobes of the prostate that differ in both their morphology and their biochemical characteristics is extremely variable among different species.

(5) Prostate enzymes readily convert testosterone, the principal circulating androgen, into unconjugated 5α-dihydrotestosterone (DHT) and 5α-androstanediols; DHT may represent the "active form" of testosterone for many of the actions of testosterone on male accessory glands of reproduction but not on all androgen-responsive tissues.

(6) The prostate contains specific androgen-receptor proteins that have a high and specific avidity for DHT and which are implicated in the sequestration and retention of androgenic steroids in prostate cell nuclei. Certain potent anti-androgenic substances (e.g. cyproterone acetate) compete with DHT for binding sites on androgen-receptor proteins but have little influence on the enzymic reduction of testosterone to DHT. Androgen-insensitive mutants of certain mammalian species—male pseudohermaphrodites exhibiting an external female phenotype and lacking many male or female internal genital organs—may lack specific androgen-receptor proteins.

(7) The effects of estrogens on the prostate are complex and can involve (a) depression of interstitial cell stimulating hormone output via the hypothalamico-anterior hypophyseal axis: (b) direct inhibition of testosterone secretion by Leydig cells of the testis; (c) fibromuscular hyperplasia and epithelial hyperplasia or metaplasia of prostate cells. The prostate gland contains small but definite amounts of specific estrogen-receptor proteins. Estrogens are only feeble antagonists of androgen-receptor proteins.

The evidence in favor of these propositions has been amply reviewed (1–11) and will not be considered in detail here, especially because many of their features are discussed in other contributions to this Workshop. But it may be appropriate to begin with a few general features of human pros-

Investigations from the senior author's laboratory were supported by a research Grant (HD-04592) from the National Institute of Child Health and Human Development

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tate physiology that are germane to benign hyperplastic enlargement of this gland.

For technical reasons, a great deal that has been written about the biochemical characteristics of the human prostate is in fact somewhat uncertain. Thus, it is difficult to obtain large quantities of "normal" prostate tissue from healthy young men. The gland is a common site of bacterial infections that even in their mild forms may perturb certain of its biochemical characteristics. What is often designated as human "prostate secretion" is usually obtained from the urethral meatus after digital massage of the prostate via the rectum; such fluids may be contaminated with material from other glands or ducts of the male genital tract. Certainly the distinction between "resting" versus "stimulated" secretions (the latter evoked by Faradic nerve stimulation or injection of cholinergic drugs) seems to be a valid one (12,13) and should be taken into account with regards to (a) effects of hormones on prostatic secretion and (b) any consideration of possible stasis of secretions in the glandular lumina that might relate to neoplastic or other pathologies of the gland. The presence of powerful proteolytic enzymes in prostatic secretion (see below) makes analysis of bulk proteins and other enzymes in this fluid difficult because of their rapid degradation.

Carcinoma of the prostate nearly always originates in the outer region ("the prostate proper") of the gland whereas benign prostatic hyperplasia originates from the mucosal or submucosal glands ("inner prostate"). However, there seems to be no consensus as to whether the initial changes in BPH occur in the glandular epithelium or in the periglandular fibromuscular stroma adjacent to the urethra, as discussed in a penetrating review by Scott (13). Furthermore, most discussions of the actions of androgens on the prostate at cellular and molecular levels have centered around biochemical processes, and particularly macromolecular biosynthetic reactions, in epithelial cells. The extent to which the physiology of cells of the fibromuscular stroma, or even endothelial cells, in the prostate *in situ* in any species is directly controlled by androgens or other hormones is far from clear.

Another matter of debate is whether the "normal" human prostate is really comprised of two separate zones that can be easily demarcated functionally and histochemically, or in terms of their responsiveness to estrogens (1,3,14). And it remains unsettled whether there exist convincing homologies between the human prostate (or different regions thereof) and various lobes or districts of the prostate gland of other non-primate species.

Benign prostatic hyperplasia is almost exclusively a disease of elderly men. As considered extensively in this Workshop, studies on age-dependent changes in circulating gonadal and other hormones are obviously pertinent to this all too common disease. Nevertheless, although there seems to be a central endocrine component to the genesis of BPH, as evidenced by the classic studies of Huggins (11), it must be emphasized that benign or malignant tumors of adjacent human male genital glands such as the seminal vesicles are very rare indeed. It is therefore extremely unlikely that alterations in the levels of various hormones in blood with advancing age represent the *only* major factors in the etiology of BPH. Among the many other agencies that need to be considered in this regard are: genetic features of intrinsic aging processes in various types of cells in the prostate, the effects of bacterial or viral infections, pharmacologic interferences, the degree of nervous stimulation of prostatic secretion and its discharge into the urethra, and all sorts of immunological parameters.

The wide attention given to the actions of steroid and protein hormones on prostate growth and development by others in this Workshop makes it unnecessary for us to consider these subjects here. It also seems pointless to provide a litany of every one of the known biochemical characteristics of the human prostate that may conceivably be germane to BPH. Rather this article deals only with five selected features of the chemical physiology of the prostate that in our view are worthy of more intensive scrutiny in relation to prostate neoplasms.

3′,5′-Cyclic Mononucleotides

The Workshop testifies to innumerable ongoing investigations on steroid hormone receptor proteins in relation to hypertrophy and hyperplasia of prostate cells. The essence of current views on the role of androgen and other steroid receptor proteins in the regulation of responsive tissues is that the hormones (or their active metabolites) combine with such receptors in the cytoplasm, following which the cytoplasmic hormone-receptor complex undergoes a transformation (perhaps a conformational change) and then is captured by the cell nucleus where the complex is retained in association with chromatin, so as to influence RNA transcription or other intranuclear events (it is possible that the hormone-receptor complexes also directly regulate certain cytoplasmic biochemical events). In other words, by interacting with specific receptor proteins inside the cytoplasm and nucleus of target cells, the steroid hormones exert their actions in-

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tracellularly. In contrast, there is strong evidence that many protein, polypeptide, and catecholamine hormones do not work by such mechanisms, but rather are recognized by and combine with specific receptor macromolecules located on the outside of cell membranes (and in some instances on nuclear membranes as well). Such hormonal interactions lead to altered synthesis of adenosine-3′5′-cyclic monophosphate (cAMP) by adenyl cyclase(s) apparently located on the inner surface of cell membranes. The cAMP can then act as a "second messenger" to amplify the initial hormone-receptor interactions by all sorts of processes that do not require the presence of the hormone and in which cAMP-dependent protein phosphokinase reactions play an important part. This mode of hormone action does not, in principle, require that the hormone ever enters responsive cells.

Many studies have recently been carried out on the perturbation of cAMP levels and related biochemical reactions in androgen- and estrogen-dependent tissues, including the rodent prostate, as reviewed by Singhal and Sutherland (15). Injection of castrated rats with cAMP together with theophylline (an inhibitor of cAMP diesterase that destroys cAMP intracellularly) can result in increases in the wet weight and the activity of certain glycolytic enzymes in the prostate gland. The dibutryl analog of cAMP was more active than cAMP in this respect; the enzyme changes were comparable in magnitude with those evoked by treatment with testosterone propionate. It has also been reported that the formation of cAMP from labeled adenosine by rodent prostate tissue *in vitro* is enhanced by nanomolar concentrations of DHT. However, there are serious doubts that androgens act on the prostate or other male secondary sexual tissues *primarily* via mechanisms involving cAMP as a "second messenger". To give but one example of evidence against this concept, Mangan, Pegg and Mainwaring (16) reported that although they could confirm the finding of Singhal (15) that treatment with exogenous cAMP increased the activity of certain glycolytic enzymes in the prostates of orchiectomized rats in a manner comparable to testosterone, the declines in the activity of RNA polymerases and polyamine biosynthetic decarboxylases in rat prostate that can be reversed by testosterone were not influenced by cAMP. Moreover, the androgen-antagonist cyproterone does not antagonize the actions of cAMP on prostate glycolytic enzymes, and cAMP does not evoke the alterations in prostatic epithelial cell height and other morphological changes characteristic of testosterone action (16). Perhaps the effects of exogenous cAMP on the prostate are largely confined to the fibromuscular stroma.

Nevertheless, much more remains to be learned about the role of adenyl cyclase and cAMP-dependent enzymes in the prostate, particularly in regard to the regulation of DNA synthesis and cell replication in this organ. In this connection, there is evidence that in some (although not all) animal cells grown in culture, elevated cAMP levels may be a prerequisite, even if not an actual cause, of contact inhibition or density-dependent inhibition of growth (17). In addition, Goldberg (18) and others have found that the related nucleotide guanosine-3′,5′-cyclic monophosphate (cGMP) may increase markedly in cells induced to divide. Goldberg (18) has promulgated a "Yin-Yang hypothesis" in which reciprocal alterations in cGMP and cAMP levels may be involved in the control of cell division through antagonistic influences on opposing cellular processes. In any event, no studies on cGMP in relation to hormonal control of prostate growth appear to have been published up to now, and more extensive scrutiny of the roles of various cyclic nucleotides in the regulation of prostate growth is certainly warranted.

Zinc

It has long been known that the prostate glands and their secretions in man and some other species are strikingly rich in zinc (9). Biochemical and endocrinologic aspects of zinc metabolism in male genital glands are considered in a recent review by Byar (19) which provides detailed documentation of the following brief summary statements. The zinc content of normal human prostate tissue is extremely variable. There are indications that zinc levels are increased in BPH (with an inverse relationship between zinc content and the proportion of fibromuscular stroma) and decreased in prostate cancer. A number of attempts have been made to use various radioactive isotopes of zinc for scintiscanning visualization of normal and neoplastic prostate tissue in situ, but the findings obtained as yet are not encouraging from the standpoint of widespread clinical applications. The application of specific zinc chelating agents has not proved successful in the therapy of human prostate neoplasms. The selective retention of zinc in prostate tissue does not seem to be accounted for by the presence of known zinc metalloenzymes. But there are indications that the prostate of some species contains specific zinc-binding proteins. Reed and Stich (20)

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isolated a zinc-binding protein from human BPH tissue. Although this isolated protein was not shown rigorously to be homogeneous (21), it seems to contain an unusually large amount of histidine; the precise structure and functions of this human prostatic zinc-binding macromolecule remain to be elucidated.

Proteolytic and related enzymes

It has long been known that prostatic and related male genital gland secretions contain a variety of exopeptidases and endopeptidases (proteinases), as well as various inhibitors and activators of these peptide bond-splitting enzymes (9,10). Recent advances in this field were recently reviewed by Zaneveld, Polakoski and Schumacher (22).

The purification and properties of proteolytic enzymes of prostatic and seminal vesicle secretions have attracted the experimental expertise of a few biochemists, although the exact number and the functions of these enzymes remains obscure. Otherwise, the proteinases and related enzymes of prostatic fluid have largely been considered as nuisances. This is because, in the first place, they contribute to the extensive autodigestion of human seminal plasma proteins; there occurs, for example, more than five-fold increases in non-protein nitrogen and profound changes in the electrophoretic profiles of bulk proteins if human seminal plasma is allowed to stand for an hour or so at room or body temperature. It may be added here that not all human seminal plasma proteins are readily digested by proteinases originating from the prostate and other male genital glands, e.g., the remarkable constancy of the high NADP-linked isocitrate dehydrogenase of human seminal plasma on standing for 24 hr at 25° (23). It is unfortunate that so little consideration seems to have been given in many recent studies on androgen and other sex hormone receptor proteins in crude extracts of prostate tissue to the intense proteolytic activities that are present in prostate extracts from several species (9,10,24). In the second place, proteolytic enzymes of prostatic origin are of clinical importance in relation to extensive fibrinolysis in certain patients with carcinoma of the prostate who exhibit a hemorrhagic diathesis (9,10,12,22). Among various suggestions for additional functions of prostatic and other proteinases in seminal plasma are: (a) involvement in the post-ejaculatory liquefaction of normal human semen; (b) a role in the negotiation of cervical mucus by human spermatozoa and seminal plasma; and (c) formation of small vaso-active polypeptides (9,10,22).

Of conceivable importance from the standpoint of cell growth regulation is the presence of plasminogen activator(s) in human prostate and its secretion besides other exoproteinases and endoproteinases. Plasminogen activators are serine proteases, inhibited by di-isopropylfluorophosphonate, that catalyze limited proteolysis of one or a few peptide bonds adjacent to arginine residues in plasminogen. This generates the active fibrinolytic protease plasmin, which very actively splits fibrinogen and fibrin but also effectively degrades a wide variety of soluble and insoluble proteins and polypeptides. Recent studies from Reich's laboratory (25) have shown that malignant transformations of animal cells by some oncogenic viruses results in greatly enhanced production of plasminogen activator, which via formation of plasmin may not only lead to breakdown of fibrinogen, fibrin, or other proteins in tissue spaces, but also may attack proteins on the outer surfaces of cells and cause cell surface alterations that could possibly affect potentialities for cell growth. Zaneveld and his colleagues (22) have recently purified two plasminogen activators from human seminal plasma that may be of prostatic origin. Although seminal plasma plasminogen activators and urokinase exhibit some immunological resemblances, they differ in some of their biochemical properties. There also are indications that male genital gland fluids contain plasminogen inhibitors or inactivators (22). Despite the fact that benign tumors have not as yet been shown to elaborate the large amounts of plasminogen activators characteristic of some malignant cells, the possibility that prostatic plasminogen activators (or other proteinases) may be related to the onset of BPH seems worthy of study.

Other peptide bond-hydrolyzing enzymes of human prostate or seminal plasma (that may, at least in part, be derived from prostatic secretion) are (i) a neutral proteinase which at one time was thought to have chymotryptic-like specificity but is now recognized to have only few properties in common with pancreatic chymotrypsin (22), (ii) tryptic-like proteinases and (iii) various exopeptidases (19,10,24). In addition, prostate lysosomes contain acid cathepsins and aminopeptidases (3). None of these proteolytic enzymes have been rigorously characterized either structurally or functionally.

The post-ejaculatory clotting of semen in some rodent species involves the coagulation of a basic seminal plasma protein from vesicular secretions by a prostatic enzyme (26). Recent experiments indicate (27) that this semen clotting enzyme may

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be a transamidase that catalyzes the formation of interchain γ-glutamyl-ε-lysine cross links between the basic seminal vesicle secretion protein so as to render it very insoluble. The enzymic semen coagulation system thus resembles the formation of insoluble fibrin by the action of Factor XIII_a (fibrinoligase) of the blood clotting system. However, a transamidase that is very active in the anterior prostate (coagulating) gland of the guinea pig differs in several of its properties from Factor XIII_a and from the comparable guinea pig liver transglutaminase (28). Transamidases not only catalyze hydrolysis of certain glutaminyl groups in proteins as well as the formation of interchain γ-glutamyl-ε-lysine bonds in certain proteins but also incorporate many low molecular weight amines (and particularly putrescine) into proteins via covalent linkages with γ-glutamyl residues derived from peptide-bound glutaminyl residues (29). This could conceivably bear on the functional significance of aliphatic diamines and polyamines that we shall now consider. However, the transamidases of human prostate and its secretion have not been studied.

Spermine and related aliphatic polyamines.

The prostate gland and its secretion represent the richest source of the aliphatic polyamine spermine in the human body (30–32). Spermine together with spermidine and putrescine are three aliphatic amines that are present in virtually all nucleated eukaryotic cells. Although the human prostate, as well as the ventral and dorsolateral lobes of the rat prostate (33), contain strikingly high concentrations of spermidine and spermine in their epithelial cells and secretions, different lobes of the prostate in many other mammalian species, or other male genital glands such as the seminal vesicle in all mammals that have been examined heretofore, contain no more spermidine and spermine than is found in the majority of tissues. Putrescine accumulates in the prostate and other mammalian organs to a much lower extent than do spermine and spermidine.

The enzymic basis of the biosynthesis of polyamines in animal tissues was first worked out with regard to the rat ventral prostate. It involves the action seriatim of four soluble cytoplasmic enzymes: (i) ornithine decarboxylase, catalyzing the loss of CO_2 from L-ornithine to form putrescine; (ii) S-adenosylmethionine decarboxylase; (iii) spermidine synthase, which adds an aminopropyl moiety from decarboxylated adenosylmethionine to putrescine to yield spermidine; (iv) and an analagous spermine synthase that reacts spermidine with decarboxylated adenosylmethionine to form spermine. Ornithine and adenosylmethionine decarboxylase are unique in that their rates of turnover (half lives of less than 1 hr) in animal cells including the prostate are more rapid than those of any other enzyme. The activity of ornithine decarboxylase (and in some instances of adenosylmethionine decarboxylase as well) rises as much as fifty-fold in many tissues that are in the early phases of rapid growth in response to countless hormonal, pharmacologic or other stimuli. These and other features of polyamine biosynthesis, turnover and functions are reviewed in contributions from this laboratory (30–32) and in a comprehensive treatise by Bachrach (34).

Normal human semen contains spermine, largely derived from prostatic secretion, in concentrations between 1 and 10 mM or greater (30–35). Spermidine and putrescine are present in prostatic fluid or seminal plasma only in concentrations below 0.3 mM. The extracellular functions of seminal spermine are a matter of conjecture. Although statistically significant correlations between human seminal spermine levels and both the total sperm count and spermatazoan motility have been reported (35) and despite observations on the "activating" effects of spermine on human and rabbit spermatozoa (30,32), it is uncertain whether seminal spermine plays any important role in human fertility. Other conceivable extracellular functions of spermine in human reproduction are: (i) depression of bacterial growth in the prostate and urethra; (ii) regulation of semen clotting by influences on transamidating enzymes involved in this process; (iii) an aid to the negotiation of cervical mucus by spermatozoa and seminal plasma. The hypotheses are discussed in detail elsewhere (32); the available evidence for or against them is insufficient to judge their validity.

Striking influences of polyamines on intracellular macromolecular biosynthetic reactions have been reviewed extensively (30–32,34). The direct addition of spermidine or spermine in concentrations equivalent to those that occur intracellularly can influence a variety of macromolecular biosynthetic processes in cell-free systems from a variety of tissues and particularly RNA polymerase reactions directed by naked or chromatin-bound DNA. The in vitro effects of the polyamines may be positive or negative, depending on their concentrations and on the experimental conditions. The direct activation of nuclear RNA polymerase reactions by spermidine or spermine has been demonstrated with rat

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prostate preparations, and an early enhancement of certain nuclear RNA synthetic reactions occurs within an hour after treatment of castrated rats with androgens. Early increases in ornithine and adenosylmeththionine decarboxylase activities are also demonstrable in prostate following injection of androgens into orchiectomized rats, with later increases in the accumulation of spermidine and spermine. Are androgen-induced elevations in prostatic polyamine biosynthesis causally related to increases in nuclear RNA synthesis in this organ? The weight of current evidence speaks against this possibility, although none of the pertinent experiments are definitive. Besides, polyamines affect many other processes related to gene expression mechanisms, including stabilization of ribosomes and biological membranes, protein synthesis by polyribosomes, tRNA methylation and inhibition of nucleolytic enzymes (31,32,34).

Increases in polyamine levels, ornithine decarboxylase, and especially spermidine/spermine ratios often occur in many normal and cancerous tissues that are rapidly proliferating (30–32,34,36). And some of the multiple forms of DNA polymerases in certain animal tissues have been found to be directly activated by fairly low concentrations of polyamines (34,37). During the hyperplastic phase of androgen-induced rat ventral prostate growth there occur immense yet transient increases in the "replicative" DNA polymerase activity of cytosol preparations from this tissue (38). Unpublished experiments by B. Tadolini, J. Wilson and H.G. Williams-Ashman have shown that rat prostate cytosols contain another enzyme (which differs in many of its properties from the major soluble "replicative" DNA polymerase) that catalyzes the polymerization of the deoxyribonucleotide dTTP in the presence of the ribopolynucleotide poly(A) as template with oligodeoxythymidylates as initiators. The activity of the latter enzyme catalyzing the aforementioned "reverse transcriptase"-like activities is much greater than that of the "replicative" DNA polymerase in cytosol preparations from the resting adult ventral prostate of androgen-replete rats. However, neither type of prostatic deoxyribonucleotide polymerizing enzyme is enhanced directly by addition of low concentrations of spermidine or spermine, which are differentially inhibitory as the concentrations of the polyamines are raised over the 1–10 mM range.

The recent finding of the Kornberg group (39) that spermidine has profound effects on bacterial multienzyme systems that replicate single-stranded viral DNA templates and which relate to the actions of the so-called "DNA unwinding protein", provide further impetus for additional studies on the interaction of polyamines with DNA replicating systems of animal tissues, such as the prostate.

Spermidine and spermine are known to be oxidized by a variety of animal, plant and bacterial enzymes (30,34). Among the products of such reactions (which vary with the enzyme source) are very reactive aldehyde derivatives of spermidine and spermine that can also break down to the reactive unsaturated aldehyde acrolein. Polyamine oxidizing enzymes are found, for example, in the blood plasma of ruminants (34) and also in human seminal plasma (34,40). The polyamine aldehyde oxidation products and also acrolein are toxic to many types of animal cells including various malignant tumors and also spermatozoa. These reactive aldehyde derivatives of polyamines can irreversibly react with DNA and nucleotides to form covalently linked adducts (34). If such reactions occurred within prostate cells in vivo, as a result of the action of either endogenous or bacterial polyamine oxidizing enzymes, they could conceivably perturb the normal regulation of DNA synthetic mechanisms in the gland and might even be germane to malignant transformations.

Now that reliable ultramicromethods for estimation of polyamines in urine, blood serum and other body fluids are available, it would be of interest to examine the levels of spermidine and spermine in prostatic fluid, blood and urine of patients with BPH and prostate cancer, considering recent pointers to the value of such measurements in certain neoplastic diseases (41).

Corpora amylacea

The presence of corpora amylacea or prostatic calculi in the prostate gland of man and certain other species has been known for a long time (9,42). They seem to be composed of an acidic mucoprotein matrix plus some neutral polysachharides; the matrix is probably secreted by prostate epithelial cells. These bodies undergo calcification, human prostatic fluid being remarkably rich in calcium(9). The number of corpora amylacea in the human prostate increases with age (42). The relationship of corpora amylacea to the development of BPH does not seem to have been explored in depth.

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DISCUSSION

J. A. THOMAS: I would like to call your attention to the fact that at the Federation Meetings (Zepp et al., Fed. Proc. 33: 521, 1974) last year we did do some studies on cyclic G in the rodent prostate and found that it was less responsive than cyclic A to castration and androgen administration. I might add that a number of cholinergic drugs influence cyclic G in accessory organs of reproduction (Zepp and Thomas, The Pharmacologist 16: 310, 1974).

P. OFNER: As you pointed out, the AMP story is incomplete. I wonder whether you or others could comment on the possible role of cAMP in androgen-mediated events other than proliferation, such as its possible mediation of secretion by those androgens that are not bound specifically to cytosol and nuclear receptors.

W. I. P. MAINWARING: The work that we did with cyclic AMP was restricted to measurements of enzymes thought to be good indicators of the androgenic response, and we selected seven enzymes, including those that have previously been shown to change with cyclic AMP treatment. Of these, the one group that was sensitive to cyclic AMP could not be blocked with antiandrogen. In answer to your question about secretion, we did not look at it specifically. All I can say on that topic is that cyclic AMP is unable to mimick the effects of testosterone in terms of gross morphology of the prostate gland.

G. WILLIAMS-ASHMAN: May I say in reply to Dr. Ofner that I think that as so often happens in molecular biology, things run very much in fashions; currently there is a tendency to believe that there's not much relationship between cyclic mononucleotides and androgen induced prostate growth. However, I believe the surface of this story has just had a few nicks scratched in it and a lot more work should be done.

I. LASNITZKI: Our organ culture studies with cyclic AMP in which we used histological criteria seem to support Dr. Mainwaring's results. Unlike testosterone, cyclic AMP neither maintained epithelial growth nor stimulated secretion.

S. G. KORENMAN: Considering the relationship between the adenyl cyclase mediated events and the steroid mediated events, the tendency seems to be that we talk about the two sets of events going along a pathway and whether A causes B. It seems more reasonable to look at the cell as a type of tissue in which the two sets of events are simultaneous and that they modulate each other. If we look at it that way, we can understand parallelisms and antagonisms of effect. There are steriod mediated influences on the consequences of adenylate cyclase activation. Furthermore, it is important to know the catecholamine status of the tissue when studying steroid mediated events. Finally, tissue component relationships are important.

WILLIAMS-ASHMAN: I agree that some of these reactions may be pertinent to the stroma. Perhaps Dr. Hechter would like to make a comment.

O. HECHTER: The possibility that cyclic nuc-

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leotides participate in steroid hormone action has received attention but has not been definitely resolved, particularly with respect to cyclic GMP. We, as well as others, have examined the possibility that cyclic AMP participates in androgen action upon the rat ventral prostate. We find, using a variety of methods, that neither testosterone or 5α dihydrotestosterone (DHT) increases the cyclic AMP level in isolated prostate tissue fragments, incubated in the presence of the potent phosphodiesterase inhibitor (methyl isobutyl xanthine). Under these same conditions, β-catecholamines, prostaglandins, and certain peptides (e.g., ACTH and MSH) produce marked increases in cyclic AMP levels in isolated prostate. Our attempts to detect stimulatory effects of DHT or DHT-receptor complexes upon adenylate cyclase activities of various subcellular fractions derived from rat ventral prostate hemogenates have likewise been negative. Thus, our findings in accord with others indicate that androgens do not act directly to increase prostate cyclic AMP. In other studies, we examined the effect of cyclic AMP and cyclic GMP upon isolated prostate nuclei measuring overall RNA synthesis (in the presence and absence of α-amanitin) as well as the overall phosphorylation of nuclear components, likewise with essentially negative effects. In these studies, we tested purified prostate nuclei, both before and after treatment with the detergent NP-40, which removes the outer nuclear envelope and *all* of the adenylate cyclase activity associated with prostate nuclei. Thus the negative results obtained with exogenous cyclic AMP cannot be attributed to nuclear production of endogeneous cyclic AMP, sufficient cyclic AMP dependent steps involved in nuclear phosphorylation and/or RNA synthesis. The available evidence, thus, indicates that cyclic AMP plays a minimal role, if any, in the early events associated with sex steroid hormone action. The situation with cyclic GMP is different. As you know, Nelson Goldberg has been an articulate and vigorous protagonist for the view that cyclic GMP in association with calcium plays an important regulatory role, antagonistic to cyclic AMP, in cell function particularly with respect to control of proliferative phenomena. One of the striking finds which has emerged is the report of Kuhel, Goldberg and their associates that estradiol increases cyclic GMP levels in the castrate rat uterus, suggesting that cyclic GMP may well be involved in the action of sex steroids. In our studies, we find inconsistent effects of testosterone upon cyclic GMP levels in isolated rat ventral prostate fragments. While testosterone (10^{-7} to 10^{-9}M) does increase cyclic GMP levels in some experiments, dose-dependent steroid effects are not obtained consistently. Thus the question of a possible role for cyclic GMP in androgen action in prostate remains open and needs to be systematically explored. At the present time, the cyclic GMP field is in a state of flux. An increasing number of experiments suggest that many agents which increase cyclic GMP levels in target tissues do so by way of a primary action on calcium entry into the cell, the increased cytoplasmic Ca^{2+} serving to activate cytoplasmic guanylate cyclase. These studies have served to expand the importance and generality of a "second messenger" role for Ca^{2+}, but do not lend support to the idea that cyclic GMP, like cyclic AMP, is directly involved in the hormonal response sequence.

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ANDROGEN TRANSPORT AND METABOLISM IN THE PROSTATE

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Introduction

The mechanism of androgen action in responsive tissues is an unresolved problem. Part of the difficulty derives from the fact that androgens are converted to several types of metabolites in target tissues and that these metabolites have physiological actions of their own. In certain cases a metabolite may act synergistically with the parent hormone while another product may have inhibitory or antagonistic effects. The problem is further complicated by the fact that some metabolites act only in the cells in which they are formed whereas others gain entry into the circulation and function hormonally at another site. Finally, differences in the pathways of metabolic transformation and in the regulation of these pathways exist between different responsive tissues of the same animal and in identical tissues from different species. Formidable barriers thus exist to a complete understanding of androgen physiology. This review will concentrate on androgen metabolism in a single tissue, the ventral prostate of the rat. It is our hope that observations made in this tissue will provide information of value for the future construction of a cohesive picture of androgen metabolism and action in the entire androgen responsive system of the intact organism.

Androgen Entry Into the Cell

The precise mechanisms whereby steroid hormones gain entry into cells are difficult to formulate (or even to examine) in terms of transport physiology, since the hormones exist in the cell in both bound and free forms, either of which may be important in controlling entry into the cell and in exerting physiologic effects. Nevertheless, on the basis of evidence at hand, it is possible to suggest a reasonable working model to explain both the entry process itself and the known development of tissue gradients (Fig. 1).

Although the mechanism of entry may be more complex than simple passive diffusion through the plasma membrane, indirect experimental evidence suggests that the entry process is not energy dependent and that free steroid rather than a steroid-protein complex enters the prostatic cell. This interpretation is based on four types of observations: First, in organ culture androgen uptake is inversely related to the concentration of serum binding proteins in the incubation media (1,2). Second, the androgenicity of steroids correlates with the concentration of free rather than total steroid in plasma (3). Third, pulse-chase experiments in the rat (4) and kinetic studies in the perfused canine prostate (5) support the concept of a passive diffusion mechanism at the plasma membrane. Finally, the concentration of testosterone in the prostate of man (6), dog (7), and rat (8) is lower than that of plasma, a finding consistent with transport by passive diffusion.

The situation is more complex when dihydrotestosterone is considered. Dihydrotestosterone concentration is higher in the prostate than in plasma, and, in fact, the total concentration in prostate of the two 17-hydroxysteroids (testosterone plus dihydrotestosterone) is also in excess of that in plasma. At least two factors are thought to play a major role in the development of this "gradient" in

The original work described in this review was supported by the U.S. Public Health Service Grant AM03892.

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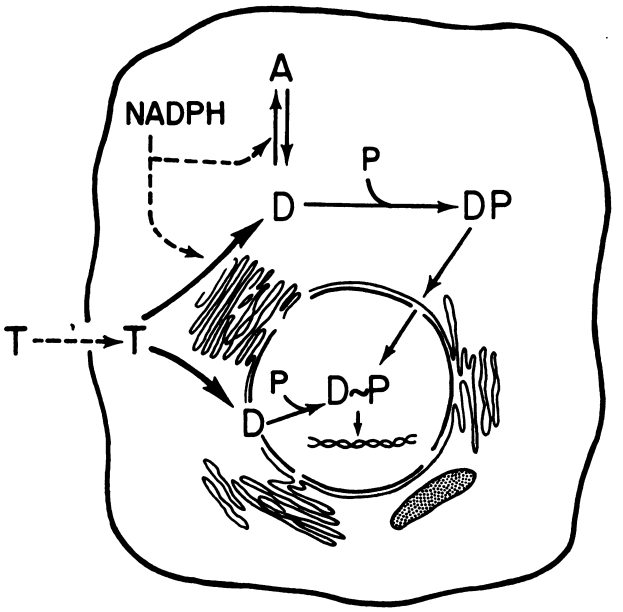

FIGURE 1. A schematic model illustrating the interrelationship of testosterone (T) metabolism via the 5α-androstane pathway to dihydrotestosterone (D) and 3α-androstanediol (A) and the specific interaction of dihydrotestosterone with cytoplasmic or nuclear receptor proteins (P) in facilitating the passive entry of testosterone into the prostatic cell.

androgen concentration between blood and cells. First, after entry into the cell, testosterone is reduced to dihydrotestosterone by the 5α-reductase enzyme; this process serves to keep the intracellular concentration of testosterone low and thus promotes the continued diffusion of testosterone down an activity gradient into the cell. Second, dihydrotestosterone, once formed, is bound to a variety of cellular proteins. While the entry event is not itself energy dependent, it should be noted that the development of significant intracellular androgen gradients does depend on the availability of reducing equivalents of NADPH. The latter most likely comes from glucose oxidation via the pentose phosphate pathway. The net effect of this process is the intracellular accumulation of significant amounts of the protein-steroid complexes that are presumed to be the physiological mediators of androgen action.

Transport events within various compartments of the prostatic cell are less clearly understood. Aside from their putative indirect role in the control of steroid uptake across the plasma membrane of the prostatic cell, the specific cytosol receptor proteins probably also function as transport proteins within the cell. The fact that soluble cytoplasmic androgen receptors bind to nuclear chromatin suggests that the cytosol receptor-dihydrotestosterone complex might be translocated directly into the nucleus where transcriptional events are initiated. Several lines of evidence are compatible with such an interpretation. Rennie and Bruchovsky (4,9) demonstrated a temporal dissociation in the labeling of cytosol and nuclear receptors when rats were injected with radioactive dihydrotestosterone, the former becoming labeled first, and a reciprocal relation between bound hormone in the cytosol and nucleus; i.e. decreases in cytosolic radioactivity could be accounted for by the increase in receptor-associated radioactivity in the nuclear fraction. Recent direct evidence idicates that ^{131}I-labeled receptor-dihydrotestosterone complex of prostate cytosol is efficiently incorporated into the non-histone protein component of chromatin (10). The types of forces involved in the formation of the various sizes of protein-steroid complexes and in the movement of these complexes into the nucleus are largely undefined, although it is likely that both processes are fundamentally passive.

Finally, the ability of prostatic tissue to secrete metabolites (such as dihydrotestosterone) into the prostatic secretion (11) and into venous blood in amounts sufficient to cause significant arterio-venous concentration gradients (12) has been demonstrated. These findings imply that additional transport fates exist for at least one metabolite of testosterone, and the suggestion has been made that two pools of steroids exist within the prostate, one available for metabolism and the other available for storage or disposal by secretion (12).

In summary, if one assumes that the empirical function of androgen transport is to develop some critical intracellular concentration of androgen-protein complex, then a three step mechanism involving initial passive diffusion, metabolism, and steroid binding provides a satisfactory explanation for the development of significant intracellular concentrations of the active hormone-protein complex.

Androgen Metabolism Within the Cell

The metabolism of androgens in target tissues occurs by several metabolic pathways. The best characterized pathway in prostate is that involving formation of the 5α-androstanes which in turn are believed to serve as the primary intracellular mediators of androgen action.

The two principal enzymatic reactions involved in the formation of physiologically active 5α-androstanes are summarized in Fig. 2. Several

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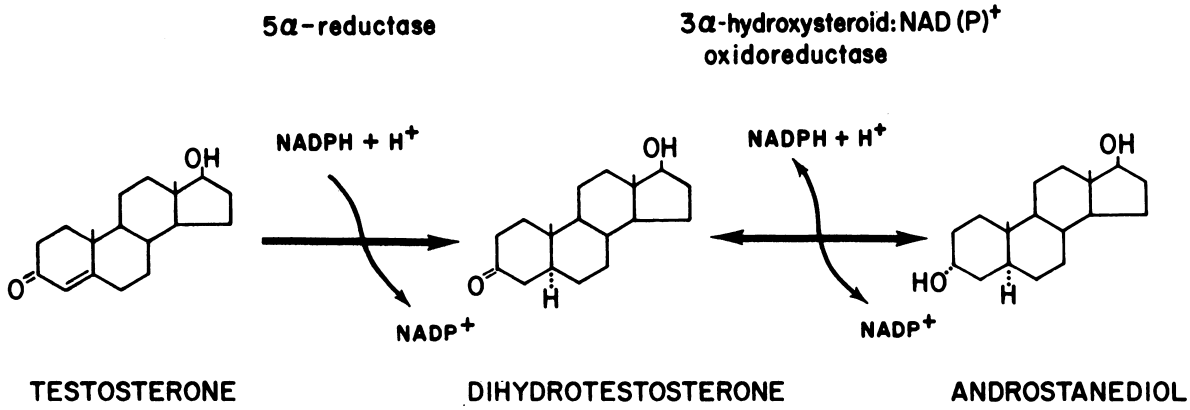

FIGURE 2. Two principal enzymatic reactions involved in the 5α-androstane pathway in rat ventral prostate.

features of the over-all pathway deserve comment. The initial reaction, namely the 5α-reduction of testosterone, has been assumed to be irreversible, although it has been recently reported that the process is reversible in liver if oxidized coenzyme Q is used as electron acceptor (13). The implications of this finding for prostate physiology must await further study. The second reaction is catalyzed by 3α-hydroxysteroid oxidoreductase and involves the reversible oxidation and reduction of the 3-keto group. Like the 5α-reductase, the process utilizes NADPH preferentially. The products of these reactions, 3α-androstanediol and dihydrotestosterone, are, as mentioned above, more potent androgens in prostate than is testosterone itself.

It should be emphasized that at least two other pathways of metabolism may be of importance in this tissue, although their quantitative and physiological significance is less well understood. The 17β-hydroxysteroids shown in Figure 2 may be interconverted to the corresponding 17-ketosteroids by the 17β-hydroxysteroid dehydrogenase enzyme. While the products of this reaction (androstenedione, androstanedione, and androsterone respectively) are only weak androgens, the 17-oxidoreductase pathway could be important in regulating the steady state levels of the active androgens, a possibility that deserves serious study. In addition, since testosterone (but not 5α-androstanes) can be converted to estrogen in many peripheral tissues, the aromatase reaction sequence also needs to be examined in prostate. It should be emphasized that the capability for estrogen formation has not yet been demonstrated in this tissue, but if present it might serve a critical function in over-all androgen action.

5α-Reductase. The formation of 5α-dihydrotestosterone by prostatic slices was demonstrated initially by Farnsworth and Brown (14), and the presence of an enzyme catalyzing the reaction in homogenates of prostate was first reported by Shimazaki et al. (15). The observation by Bruchovsky and Wilson (16) that [^{3}H]dihydrotestosterone is the major steroid found in prostatic nuclei following the administration of [^{3}H]testosterone to intact animals indicated that this pathway of metabolism might be of physiological significance and stimulated interest in the 5α-reductase enzyme. Studies in our laboratory have focused on the properties of this memrane-bound enzyme, its subcellular localization, and the regulation of its activity (17,18).

The substrate specificity of the reaction is shown in Table 1. Six steroids have an equal or greater capacity to serve as a substrate for the enzyme than does testosterone (19). For example, the apparent Kms for 20α-hydroxy-4-pregnene-3-one, epitestosterone and progesterone are considerably lower than that for testosterone, whereas the values for cortexolone, androstenedione, and deoxycorticosterone were approximately the same as for testosterone. Steroids of the C_{21}-11-oxo-series (corticosterone, cortisone and cortisol) are inactive as substrates for the prostatic enzyme in contrast to the situation in liver where they are potent substrates for the 5α-reductase enzyme(s). These findings indicate that the prostatic 5α-reductase has a distinct substrate specificity for Δ^4-3 keto steroids lacking oxygen groups at carbon 11.

The intracellular localization of the 5α-reductase in the rat prostate is of interest. Studies in several laboratories (19–23) have demonstrated that nuclei, isolated by sedimentation through high molar sucrose, contained 25–60% of total cellular 5α-

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TABLE I.—Efficiencies of various C_{19} and C_{21} steroids as substrates for the 5α-reductase relative to testosterone

Steroid	Relative efficiency as substrate
20α-Hydroxy-4-Pregnene-3-one	4.3
Epitestosterone	2.8
Progesterone	2.3
Cortexolone	0.8
Androstenedione	0.7
Deoxycorticosterone	0.7
Corticosterone	0
Cortisol	0
Cortisone	0

The experimentally measured Km values for various Δ^4-3 keto-steroids (ref. 19) have been recast utilizing the reciprocal of the Km value and normalized to give testosterone a value of 1.0. Values above and below 1.0 represent substrate efficiencies that are increased and decreased, respectively, relative to testosterone.

reductase (Table II). However, since nuclei prepared in this manner are known to be contaminated with varying amounts of endoplasmic reticulum and since the enzyme is also present in the latter fraction, the possibility existed that the apparent nuclear localization might be an artifact. To settle this issue, two additional types of experiments were conducted.

In the first, the nuclear localization of 5α-reductase was compared with that of 4 other enzymes known to have specific localization in other cell membranes (Table III). In this study, 55% of total cellular 5α-reductase was recovered in nuclei under circumstances where maximum contamination with enzymes located in mitochondria, endoplasmic reticulum, and lysosomes was only 7% (24). Clearly, the nuclear localization of 5α-reductase could not be due to contamination with other cell organelles.

In the second experiment, the nuclear membranes themselves were isolated and shown to be free of significant contamination with endoplasmic reticulum (Fig. 3). Purified nuclei (A), endoplasmic reticulum (B), and a mixture of the two (C) were adjusted to a density of 1.42 by the addition of cesium chloride and introduced beneath a continuous cesium chloride gradient of density range 1.05–1.4. Following ultracentrifugation turbid bands corresponding to the membrane fractions were visible, and analysis of consecutive fractions for 5α-reductase indicated that all the enzyme activity was coincident with the turbid zones at a median density of 1.25 for nuclei and 1.20 for endoplasmic

TABLE II—Recoveries of 5α-reductase in prostrate nuclei

Investigators	Year	Reference	Method Used For Nuclear Isolation	5α-Reductase in Nuclei	
Frederiksen and Wilson	1971	19	2.2 M Sucrose	53%	a
Shimazaki, et al.	1971	20	2.4 M Sucrose	61%	b
Roy	1971	21	2.3 M Sucrose	41%	b
Nozu and Tamaoki	1973	22	2.2 M Sucrose	Majority	c
Verhoeven, Lamberigts and De Moor	1974	23	2.2 M Sucrose	25%	a

a. Corrected for the recovery of cellular DNA in the nuclear fraction.
b. Not corrected for the recovery of cellular DNA in the nuclear fraction.
c. Percentage recovery not stated.

TABLE III—Nuclear localization of 5α-reductase in rat ventral prostate

Enzyme	Major Intracellular Locus	Recovery in Nuclei per cent
5α-Reductase	Nuclei and endoplasmic reticulum	55.2
Succinic Dehydrogenase	Mitochondria	1.0
NADH Cytochrome c Reductase	Mitochondria and endoplasmic reticulum	6.4
NADPH Cytochrome c Reductase	Endoplasmic reticulum	6.9
Acid Phosphatase	Lysosomes and cytosol	1.6

The percentages of homogenate enzyme activity recovered in the nuclear fraction represent the actual recoveries, corrected for the recovery of cellular DNA (62.9%). Adapted from reference 24.

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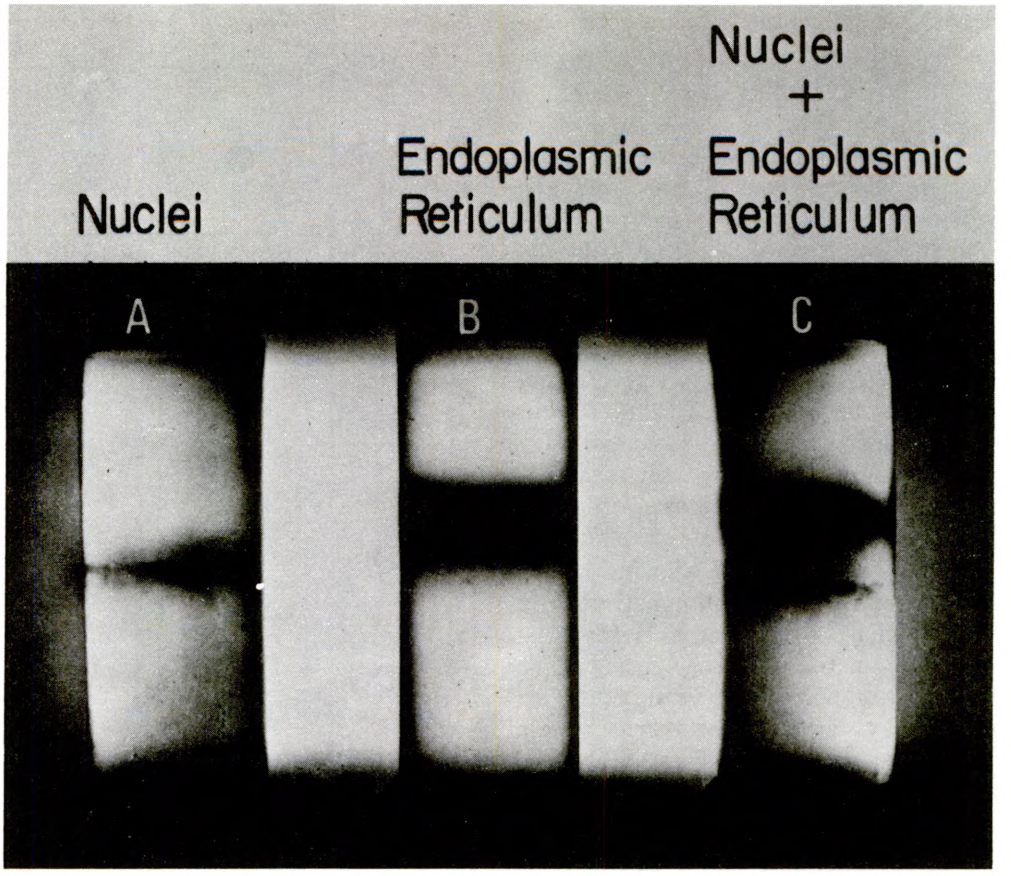

FIGURE 3. Separation of nuclear and endoplasmic reticulum membranes by cesium chloride density gradient centrifugation.

reticulum. Two discrete density classes of membranes and 5α-reductase were clearly separable in the flotation gradient in which nuclei and endoplasmic reticulum were mixed before commencement, demonstrating that any contaminating endoplasmic reticulum membranes could be separated from nuclear membranes (24). Thus, a major fraction of the cellular 5α-reductase is located in nuclei.

Solubilization and partial purification of the 5α-reductase have also been achieved. Careful titration of nuclear membranes with digitonin releases 5α-reductase into a 100,000 × g supernatant, and in the presence of 3 M KCl the predominant form of the enzyme is retained on agarose 1.5 M column chromatography (Fig. 4). Utilizing sequential extraction and agarose chromatography or density gradient centrifugation, an approximate 100-fold purification of the enzyme has been achieved. The solubilized enzyme has an apparent molecular weight of 300,000, sediments at 13–15S, has a pH optimum of 6.9, exhibits an absolute requirement for NADPH, and has an apparent K_m for testosterone of approximately 1 μM. The properties of the nuclear and microsomal enzymes appear to be

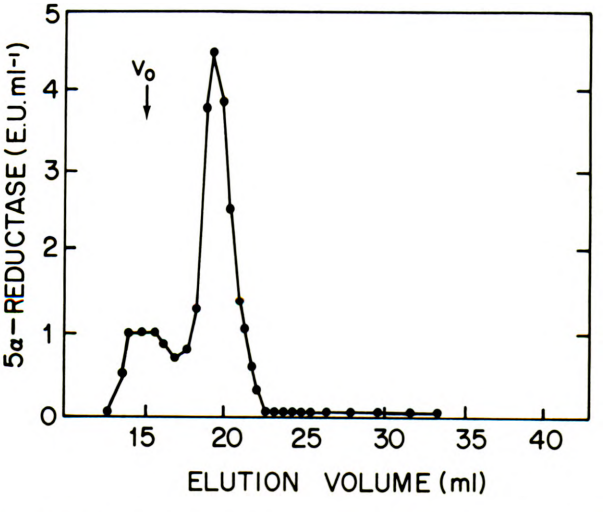

FIGURE 4. Agarose chromatography of digitonin extracts of prostatic nuclei in the presence of 3 M KCl. Biogel A—1.5m; Vo = void volume by blue dextran. Taken from Moore and Wilson (ref. 18).

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identical except that the microsomal enzyme appears to be somewhat larger as assessed by sedimentation and chromatographic characteristics (18).

The activity of the 5α-reductase of rat ventral prostate is under endocrine control. Shimazaki and co-workers demonstrated that 5α-reductase falls following castration and increases after androgen treatment (25), and the effects of other hormonal manipulations were subsequently studied in our laboratory (26), utilizing the response of the 7-day castrate rat prostate as described by Coffey, Shimazaki, and Williams-Ashman (27). The changes in concentration of prostatic 5α-reductase and prostatic weight with time following the daily administration of 2 mg testosterone propionate are summarized in Fig. 5. Data are expressed as the per cent of the 7-day castrate control. Following castration the gland atrophies; after administration of testosterone propionate this process is reversed rapidly, and striking increases in the concentration of 5α-reductase occur, the rise remaining linear for approximately 4 days and ultimately reaching a plateau in which the enzyme concentration is more than 10-fold that of the 7 day nadir. Adrenalectomy, thyroidectomy, hypophysectomy and the simultaneous administration of estradiol did not influence the 5α-reductase response to testosterone propionate in the castrate rat (26). When 12 different steroids were tested for their effect in this system, 5α-reductase activity correlated with the change in prostate weight.

3α-Hydroxysteroid Oxidoreductase. The second enzyme that has been characterized is the 3α-hydroxysteroid dehydrogenase of rat ventral prostate. At least two activities are present in prostate, a cytosolic enzyme that utilizes NADPH and a particulate enzyme that utilizes NADH preferentially (28).

The cytosolic enzyme is the more active of the two and has been studied in detail (29). In rat ventral prostate it is stereospecific, and only 3α-hydroxysteroids are formed as products (29). This was shown by incubation of cytosol with [^{3}H]-dihydrotestosterone in the presence of NADPH following which the reaction products were subjected to alumin oxide chromatography, a procedure that clearly separates 3α and 3β diols (Fig. 6). All the androstanediol radioactivity was associated with the 3α-epimer.

The substrate specificity of the enzyme is also of interest. Various 5α-reduced, 3-keto steroids and Δ^4-3-keto steroids of the C_{19} and C_{21} series were tested as substrates (S) and as inhibitors (I) of the

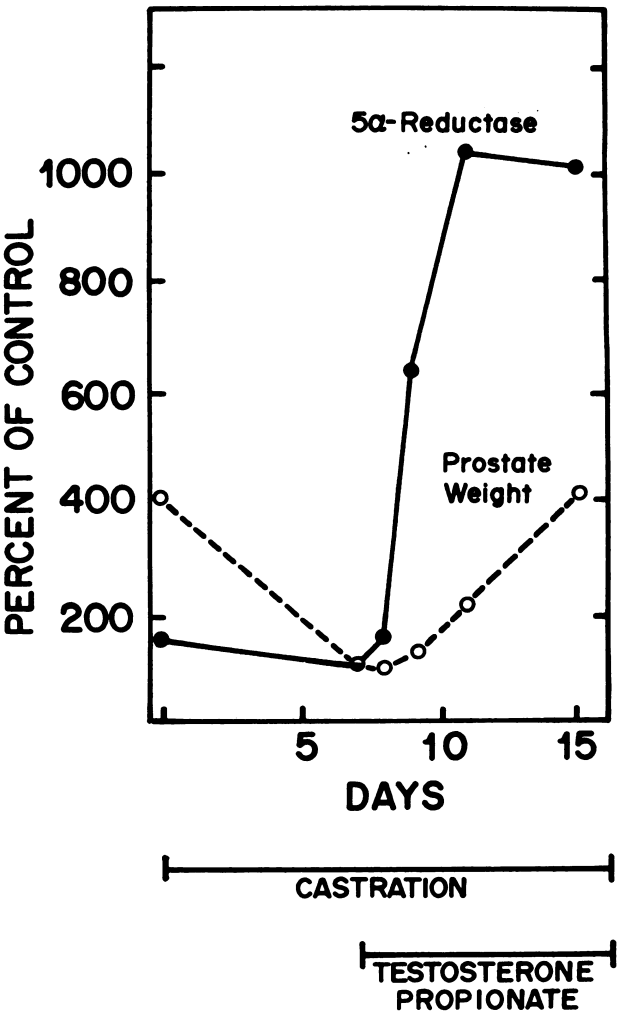

FIGURE 5. Time-sequence of changes in prostatic weight and concentration of 5α-reductase in response to a 7 day castration period followed by the daily administration of 2 mg testosterone propionate. Prostates from normals, castrates and castrates that had been treated with testosterone propionate for 1, 2, 4 or 8 days were weighed and assayed for 5α-reductase in cell-free homogenates. For each time period, groups of 2–10 rat prostates were pooled for each study. Data for weight and anzyme activity (E.U./g) represent the mean of 3–12 separate studies and have been expressed as per cent of the 7 day castrate group. Adapted from reference 26.

enzyme (Table IV). Three features of these studies deserve comment. First, a variety of 5α-reduced steroids were as good or better substrates than dihydrotestosterone and were effective competitive inhibitors, provided they did not contain the 11-oxo group. In this regard, the 3α-hydroxysteroid dehydrogenase and 5α-reductase enzymes of prostate are remarkably similar. Second, no Δ^4-3-ketosteroid tested was reduced to the correspond-

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TABLE IV—Relative efficiencies of various steroids as substrates (S) and competitive inhibitors (I) of the 3α-hydroxysteroid oxidoreductase of rat prostate cytosol

Series	5α-Reduced Steroid	Relative efficiency as S	Relative efficiency as I	Δ^4-Steroid	Relative efficiency as S	Relative efficiency as I
	Androstanedione	0.8	0.9	Androstenedione	0	0.5
C_{19}	Dihydrotestosterone	1.0	1.0	Testosterone	0	0.8
	Epidihydrotestosterone	1.5		Epitestosterone	0	0.6
	Allopregnanedione	2.6	2.2	Progesterone	0	3.1
C_{21}	Dihydrodeoxycorticosterone	2.6	3.8	Deoxycorticosterone	0	3.3
	Dihydrocortexolone	1.9	3.9	Cortexolone	0	4.5
	Dihydrocorticosterone		0	Corticosterone	0	0
C_{21},11-oxo	Dihydrocortisol		0	Cortisol	0	0
	Dihydrocortisone		0	Cortisone	0	0

Experimentally measured Km and Ki values for various Δ^4- and 5α-reduced 3-keto steroids (ref. 29) have been expressed as the reciprocal of the Michaelis or Inhibitor constant and normalized to give dihydrotestosterone a value of 1.0. Values above and below 1.0 represent efficacies as substrates or inhibitors that are increased and decreased, respectively, relative to dihydrotestosterone.

ing 3α-allyl alcohol under the conditions of these experiments. Thus, prior 5α-reduction appears to be obligatory before 3α-reduction can take place. Third, in contrast to their inability to serve as substrates several Δ^4-3-keto steroids competitively inhibit the reaction in a pattern similar to that of the corresponding 5α-reduced steroid. However, Δ^4-3-keto steroids such as corticosterone, cortisol, and cortisone are devoid of inhibitory capacity, apparently the consequence of oxygen substitution at the 11 position. To summarize, prior 5α-reduction is required for 3-keto reduction, Δ^4-3-keto steroids act as competitive inhibitors although they are not substrates, and steroids containing 11-oxygen groups are inactive as substrates or competitive inhibitors.

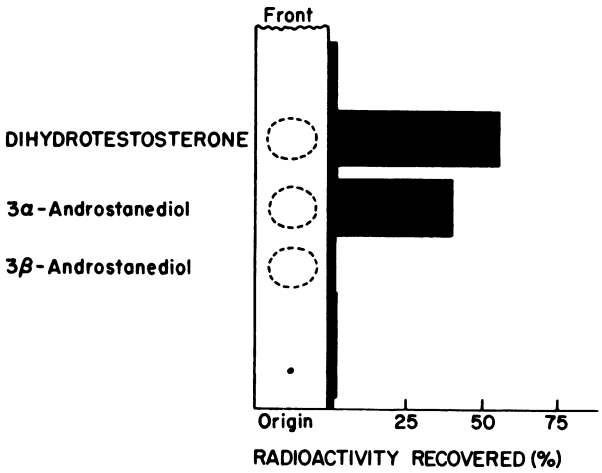

FIGURE 6. Distribution of ^{3}H-radioactivity following aluminum oxide chromatography of extracts of the reaction mixtures of rat prostate cytosol and ^{3}H-dihydrotestosterone in the presence of NADPH. Taken from reference 28.

Partial purification of the enzyme was effected utilizing ammonium sulfate precipitation, agarose chromatography, DEAE cellulose chromatography and a second chromatography on agarose (29). An over-all yield of 40% was obtained, and a purification of over 100-fold was achieved. Various properties of the enzyme are summarized in Table V. It has a molecular weight of approximately 25,000-30,000 and exhibits a broad pH optimum between 6.5 and 8.0 for the reduction of dihydrotestosterone to androstanediol. A more narrow range of 8.5 to 9.0 was found for the reverse reaction. The kinetic constants for steroid and coenzyme substrates are also shown. Studies in our laboratory (Moore, R. J., Wilson, J. D. Unpublished information) and in that of Shimazaki (30) suggest that the enzyme activity in prostate is independent of control by androgens, adrenal or pituitary hormones, and estrogens.

Perhaps the most important aspect of the enzymology of the reaction from a physiological standpoint relates to the question of its equilibrium.

TABLE V—Properties of the 3α-hydroxysteroid oxidoreductase of rat prostate cytosol

Molecular weight	25,000—30,000	
pH optima—reduction	6.5—8.0	
oxidation	8.5—9.0	
Michaelis constants	Dihydrotestosterone	0.6 μM
	NADPH	2.2 μM
	NADH	0.6 mM
	Androstanediol	4.2 μM
	NADP	5.0 μM
	NAD	1.0 mM
Independent of endocrinological control		

Adapted from Taurog, Moore and Wilson (29)

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Since the reaction is reversible, it is of importance to know whether androstanediol or dihydrotestosterone accumulates in the intact cell. For this purpose, the equilibrium constant was measured at pH 7.4 utilizing the 100-fold purified enzyme preparation (Fig. 7). Two reaction mixtures were supplied with either [^{3}H]dihydrotestosterone or [^{3}H]androstanediol and equimolar concentrations of both reduced and oxidized NADP. The conversion of dihydrotestosterone to androstanediol increased linearly with time, reaching a plateau of approximately 60%. In a separate reaction mixture, the formation of dihydrotestosterone from androstanediol increased in a reciprocal fashion. These data suggest that the equilibrium favors androstanediol formation. In the intact cell, where the NADPH/NADP ratio exceeds unity, the conditions are even more favorable for the accumulation of androstanediol. The fact that the activity of the 3α-hydroxysteroid dehydrogenase greatly exceeds that of the 5α-reductase also favors androstanediol accumulation at the expense of dihydrotestosterone.

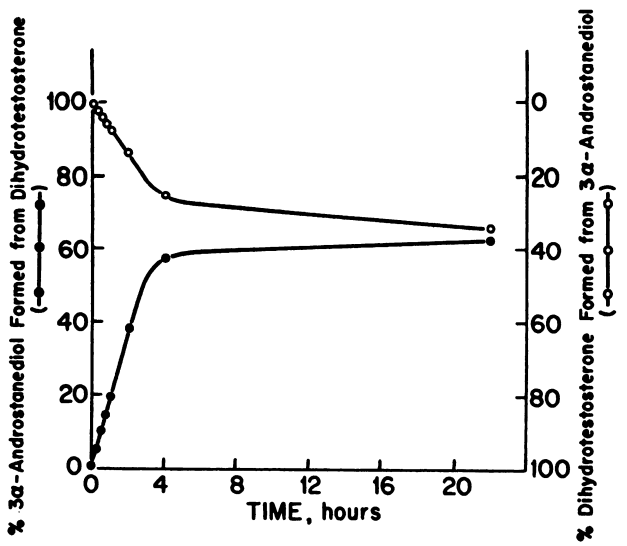

FIGURE 7. Kinetics of formation of products of the reaction catalyzed by 3α-hydroxysteroid oxidoreductase and the equilibria of independent oxidation and reduction reactions. Separate reaction mixtures contained either 0.5 μM [1,2-^{3}H]-androstanediol (4.3 Ci/mmole) or 0.5 μM [1,2-^{3}H]-dihydrotestosterone (4.3 Ci/mmole) and 0.5 mM NADPH, 0.5 mM NADP, 0.5 mM dithiothreitol and 100 μg of a 100-fold purified enzyme preparation (described in Table VI) in 0.1 M potassium phosphate pH 7.4 and a final volume of 2.0 ml. The mixtures were incubated at 37°C, and aliquots were removed at the times indicated and analyzed for the formation of dihydrotestosterone and androstanediol by silica gel chromatography as described in reference 29.

Conclusions

From studies of these two enzymes, some unique interrelationships emerge (Fig. 8). First, testosterone and other Δ^4-3 ketosteroids of the C-19 and C-21 series can be converted to 5α-reduced steroids at two sites within the cell, the endoplasmic reticulum and the nuclear membrane. 11-Oxo-steroids are inactive substrates for this reaction. Second, 5α-reduced steroids may be further reduced in the cytosol to 3α hydroxy steroids; 11-oxo-5α-reduced steroids cannot serve as substrates for this reaction. Third, Δ^4-3 ketosteroids, although not substrates, may competitively inhibit the 3α-hydroxysteroid dehydrogenase, again provided they do not contain an 11-oxo group.

These interrelationships have important physiologic implications for androgen metabolism in the cell. Since the concentration of the C-21 glucocorticosteroids exceeds that of the C-19 steroids in plasma and presumably in the cell, if specificity did not exist for this pathway, i.e. if 11-oxo steroids were not excluded, then competitive inhibition by glucocorticosteroids would almost certainly prevent the formation of the active 5α-reduced and 3α-hydroxy androgens. Thus, a unique chemical compartmentalization channels androgens into specific metabolites in the cell.

The anatomic localization of enzymes may be equally important. The total activity of cytosolic 3α-hydroxysteroid oxidoreductase is greater than that of membrane bound 5α-reductase, and the equilibrium thermodynamically favors 3-keto reduction. Therefore, it is clear that in the cytoplasm the conditions favor the formation of 3α-hydroxy, 5α-reduced steroids. In contrast, in the nucleus which is enriched in 5α-reductase and contains less 3α-hydroxysteroid oxidoreductase, circumstances favor the accumulation of dihydrotestosterone. The sum of this chemical and anatomical compartmentalization favors dihydrotestosterone accumulation in the cell compartment where it is presumed to exert its major effect.

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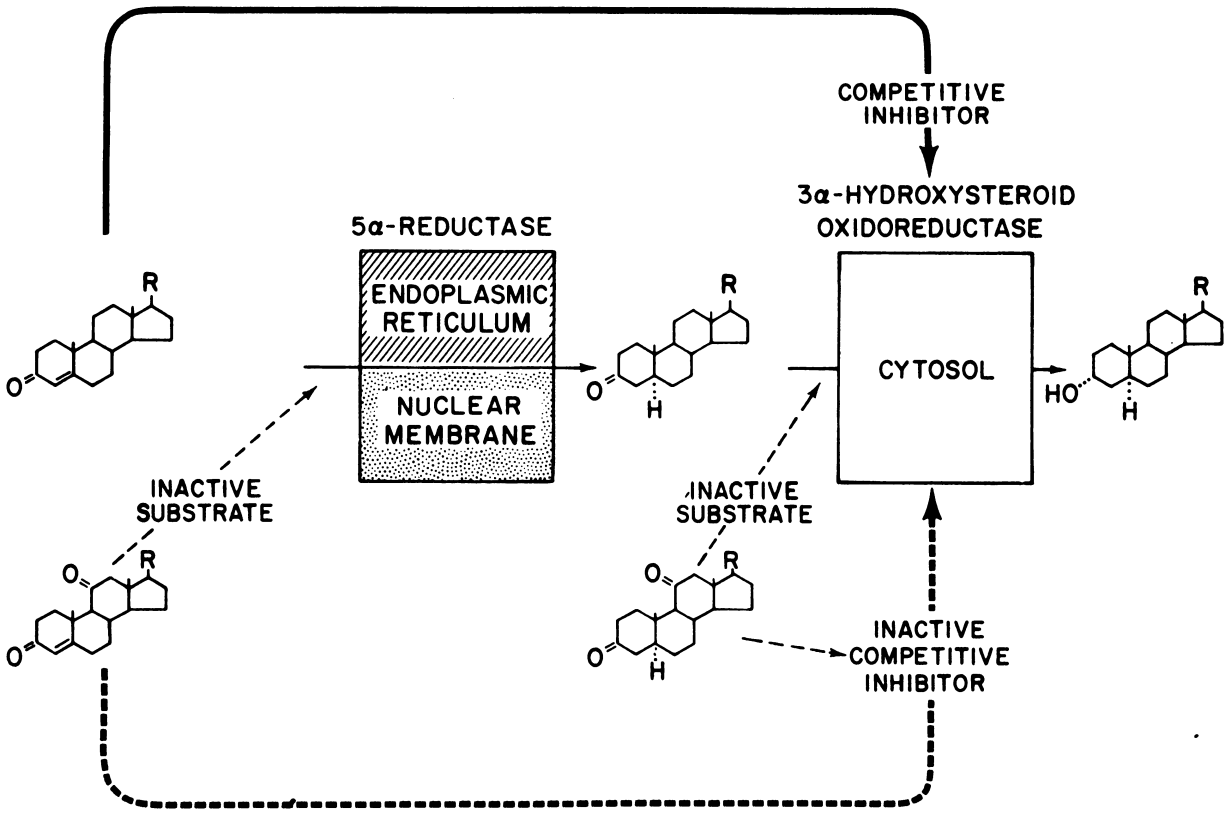

FIGURE 8. Diagrammatic representation of the anatomical compartmentalization of prostatic enzymes in the 5α-androstane pathway and the chemical compartmentalization with respect to steroids of the 11-oxo series. The notation (R) at C–17 on the structural formulae has been used to emphasize the wide variety of both C_{19} and C_{21} steroid hormones which may participate in these reactions.

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DISCUSSION

R. HORTON: Is there any evidence that the amount of cytosol binding is rate limiting in that when androgen is bound to the cytosol binding protein it is inevitably transported into the nucleus while unbound testosterone might be metabolized in the direction of androstandiol in the cytoplasm?

MOORE: The changes that I showed for 5α reductase have been evaluated both in purified nuclear to 3-alpha reduction suggests that it is perhaps the rate limiting step in transport and metabolism.

M. LIPSETT: Have you had the opportunity to see if any differential effect of androgen can be shown on the microsomal and nuclear 5α reductase, and what is the mole ratio of the steroids necessary to give 50% inhibition to the 3-alpha hydroxy steroid oxido reductase?

MOORE: The changes that I showed for 5α reductase have been evaluated both in purified nuclear fractions and microsomes, and there's a proliferation of both components of the enzyme, nuclear and microsomal following androgen administration. The Km for dihydrotestosterone is slightly less than one micromolar, and the Ki values, which would be 50% inhibition, were of the order of magnitude of 0.2 to 1 μM for the C21 steroids, deoxycorticosterone and progresterone.

O. HECHTER: When you speak about the nuclear membrane, are you speaking of the outer nuclear envelope or the inner nuclear membrane? The outer nuclear membrane may be considered as a component part of the endoplasmic reticulum; it can be "stripped" by using detergents such as triton or NP-40. Are you referring to the inner nuclear membrane?

MOORE: The nuclei have been isolated through high molar sucrose and have intact inner and outer nuclear membranes.

HECHTER: If your distribution data is correct, and I'm sure it is, some of our studies relating to the distribution of adenylate cyclase activity in ventral prostate may be pertinent. We find that prostate nuclei, purified by a modified Wilson procedure, contain only about 1–2% of the total adenylate cyclase activity present in the homogenate. If we treat purified nuclei with the detergent NP-40, we lose all of the adenylate cyclase activity; the detergent treated nuclei with inner membrane intact, contain zero activity. The adenylate cyclase activity originally associated with nuclei, thus is either an extra-nuclear contaminant or associated with the outer nuclear envelope. Our findings suggest that if the prostate nuclei in your studies were "contami-

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nated", the distribution values for 5α reductase should be small instead of approaching 50%. Your data raise the possibility that 5α reductase may be localized in the inner nuclear membrane. But if this is so, then another question arises. Why should 5α reductase be present in the inner membrane of the nucleus, to reduce a steroid, so that the metabolite formed must return to the cytoplasm to interact with a steroid receptor binding protein and then have the steroid receptor complex return to the nucleus. I have some trouble trying to understand the distribution of 5α reductase in relation to "shuttling" back and forth required to account for the overall sequence of events.

MOORE: I cannot answer that question. There is no precedent for the localization of an enzyme such as this.

N. BRUCHOVSKY: If experiments are done *in vivo* where rats are injected with increasing doses of testosterone and dihydrotestosterone, you can saturate nuclei at the same level with both steroids. Following the injection of testosterone, nuclei contain testosterone plus dihydrotestosterone. When the animals are loaded with just dihydrotestosterone, nuclei contain only dihydrotestosterone. Thus, both testosterone and dihydrotestosterone get into the nucleus when a concentration of these steroids in the cytoplasm is below 100 nanomolar. Above this concentration, almost any steroid would probably enter the nucleus. We believe that the 5α reductase in the cytoplasm regulates the ratio of testosterone and dihydrotestosterone in the nucleus.

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BIOCHEMICAL ASPECTS OF ANDROGEN RECEPTORS AND CELL STIMULATION IN THE PROSTATE

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Introduction

This article summarizes selective aspects of our recent work on the androgen receptors in rat ventral prostate and our efforts in isolating the early biochemical events that may help in the elucidation of the molecular processes of androgen action in the target cells.

Cellular Receptors for 5α-Dihydrotestosterone (DHT)

Testosterone entering the prostate cells is rapidly reduced to DHT and binds to a cytoplasmic protein (Fig. 1). Largely on the basis of findings in the laboratories of Wilson, Mainwaring, Tveter, Baulieu, and in our own laboratory, it appears that this protein (we have called it β-protein) is a specific cellular receptor that may be involved in triggering androgen action in the target cells. (Comprehensive reviews on earlier (1) and more recent work (2,3) are available).

One of the most commonly employed techniques for the detection of the DHT-receptor complex has been gradient centrifugation. In sucrose or glycerol gradient media with low ionic strength, the cytosol [^{3}H] DHT-receptor complex sediments as 8–9 S and also as 3–4 S. The 8–9 S form can be transformed to the 3–4 S form by incubation at 20–30° or by an increase of the ionic strength of the medium to 0.4 M KCl. If gradient centrifugation is to be employed for qualitative or quantitative characterization of the receptor, it should be noted that the sedimentation pattern of the androgen-receptor complex varies drastically when the pH of the medium is changed from 6.4 to 9.5 (Fig. 2). Between pH 6.4 and 7.5, most of the complex may sediment in the form of heavy aggregates, whereas, at pH 8.0 to 9.5, the complex can exist in the 6–8 S and 3.5 S forms. In the presence of 0.4 M KCl or 2 M urea, in the pH range from 6.4 to 9.5, the cytoplasmic complex sediments approximately as 3.5 S, whereas the nuclear complex is found to be at about 3.0 S.

The sedimentation property of the DHT-receptor complex also varies in media containing certain divalent metal ions. Incubation of the DHT-receptor complex with 1–5 mM $ZnCl_2$ at 0° can result in a shift of the sedimentation coefficient from 3.8 to 4.5 S. If the mixture is incubated at 20°, Zn^{++} appears to interact with and alter the receptor proteins in such a way that its affinity toward DHT is considerably reduced. As a result, the androgen is gradually released from the receptor protein.

We have also found that the cytosol DHT-receptor complex can be stabilized by ATP and GTP, indicating that an interaction of these nucleotides with the receptor protein can occur. The DHT-receptor complex appears to bind more readily to certain polynucleotides such as poly A, poly G, and poly U, than to tRNA or poly C.

The work presented in this article is supported by Grant AM-09461 from the United States National Institutes of Health and, in part, by Grant BC-151 from the American Cancer Society, Inc.

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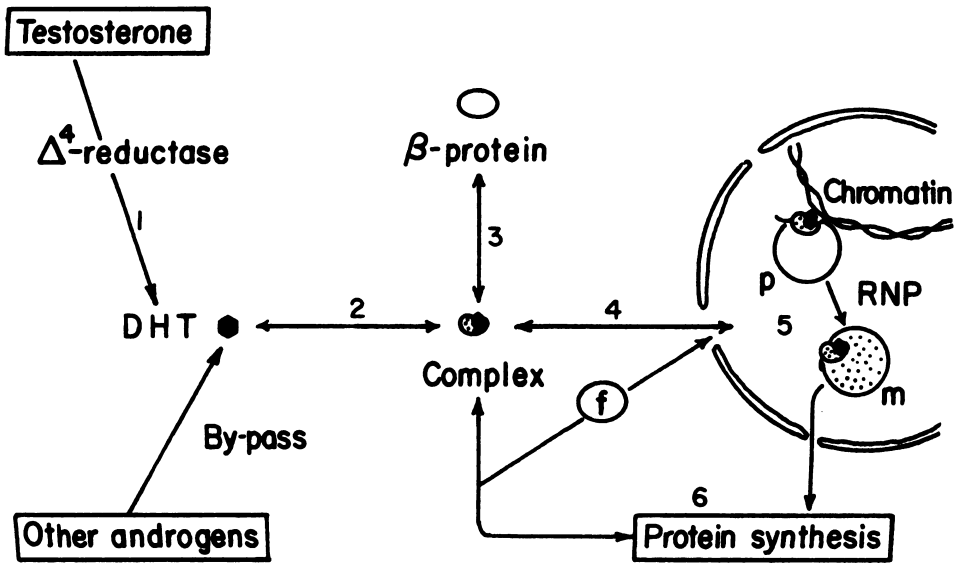

FIGURE 1. A model for the intracellular cycling of androgen receptor and the possible sites of action of antiandrogens. Some antiandrogens can act by inhibiting the formation of DHT (1). However, certain synthetic androgens, such as 7α, 17α-dimethyl-19-testosterone or 2-oxa-17β-hydroxy-estra-4,9,11-triene-3-one, can bypass such inhibition since they can bind tightly to the receptor without a prior metabolic conversion. Antiandrogens or their metabolites can also compete with DHT for receptor binding (2) or can induce alteration of the receptor protein, either directly or indirectly (3), so that the receptor cannot sustain normal binding of DHT. In some cases, this may result in the formation of an abnormal androgen (or antiandrogen)-receptor complex that cannot bind to the nuclear acceptor sites (4). Alternatively, the abnormal complex may bind to the nuclear acceptor sites, but it may either fail to exert its normal function or prevent normal complex from binding to the acceptor molecules (5). Besides having an antagonistic effect on nuclear function, some antiandrogens may impair certain cellular functions, such as protein synthesis outside the nucleus (6), or prevent cytoplasm-nuclear communication (including receptor transfer) at the nuclear membrane site. [Modified from Liao *et al.* (45), by courtesy of Plenum Press.]

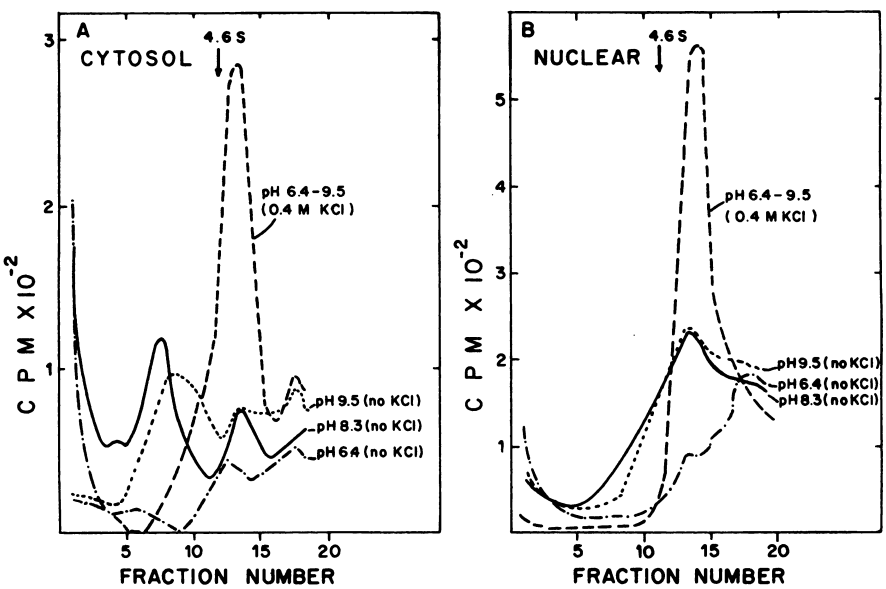

FIGURE 2. Effect of pH on the sedimentation pattern of the cytosol (A) and nuclear (B) [^{3}H]DHT-receptor complexes of rat ventral prostate. The glycerol gradient (10 to 22%) solution contained 1.5 mM EDTA, 20 mM Tris-HCl buffer at the pH shown, with or without 0.4 M KCl. Fractions were collected and numbered from the bottom of the tube. Bovine serum albumin (4.6 S) sedimented at the positions shown by the arrows.

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When the prostate microsomal fraction in rats injected with [^{3}H] testosterone is extracted with a 0.4 M KCl solution, a [^{3}H] DHT-protein complex that has a sedimentation constant of about 3 S (in 0.4 M KCl) can be obtained (1,4). The androgen-binding protein is very similar to the cytosol β-protein in its steroid specificity, heat sensitivity, sedimentation properties, and ability to be retained by prostate nuclear chromatin. The amount of microsomal DHT-binding protein can be about 30–50% of the total β-protein found in the cytosol fraction. This protein may be responsible for the retention of DHT by the microsomal fraction of prostate cells.

Various properties of the DHT-receptor complex have been described in several publications (3,5,6). These studies have been carried out with rather crude preparations, however, since the purification of the prostatic receptor has been only partially successful and only in radiometric quantities. More reliable measurements of various physical and chemical constants must await the achievement of further receptor purification.

Nuclear Receptors

The DHT receptor protein begins to disappear rapidly from the cell nuclei of rat ventral prostate shortly after the androgen is withdrawn from the animal by castration. Within minutes after the androgen is replenished, the DHT-receptor complex can be detected in the nuclei. These and our other studies involving cell-free systems (1–5) have indicated that the cytoplasmic receptor is retained by the cell nuclei only after the receptor protein interacts with and binds DHT.

The nuclear retention of the prostate DHT-receptor complex is highly steroid- and tissue-specific. Intact cell nuclei from rat liver, thymus, and diaphragm are not capable of retaining as much of the complex as are prostate cell nuclei. Various experiments have suggested that, in vivo, the cell nuclei may have a limited, but not necessarily constant number (2000—10,000 sites per diploid cell nucleus) of receptor binding (acceptor) sites. The specific binding of the androgen-receptor complex can be detected by use of a nuclear chromatin fraction (7,8). Such a study must be carried out with great care, however, as the aggregation and nonspecific association of the nuclear materials (including DNA and histone) and the DHT-receptor complex (5,7) or other steroid-receptor complexes (9–11) occur readily and yield erroneous results. Very little is understood about the acceptor molecules except that they appear to contain a nonhistone protein. (5,7).

The DHT-dependent translocation of the cytoplasmic protein to prostate cell nuclei is apparently temperature-dependent. By autoradiography (12) and by reconstruction from cellular fractions (3,5), we have observed that the nuclear retention of the receptor occurs more readily at 20° to 30° than at 0°. The temperature-dependence effect appears to involve the alteration of the DHT-receptor complex (3,6) from the cytosol form (3.5 S in 0.4 M KCl media) to the nuclear form (3 S) (2). Whether such a receptor transformation is due to a change in the conformation of the protein molecule and/or to a proteolytic action is not clear.

Androgen-Receptor Interaction

In our laboratory, we have investigated the structural requirements for binding of the steroids to the receptor protein by studying a number of androgen analogues (13) for their ability to bind to the receptor protein and to the cell nucleus. From these studies it can be concluded that the bulkiness and flatness of the steroid molecule play a more important role in receptor binding than the detailed electronic structure of the steroid nucleus. The role of Δ^4-3-keto-5α-oxidoreductase is not merely to eliminate the double bond on ring A of the steroid but rather to convert testosterone to a flatter molecule with an overall geometry that fits better to the receptor binding site. Potent androgens with conjugated double bonds extending from rings A and B to C (such as 2-oxa-17β-hydroxy-estra-4, 9, 11-trien-3-one) are indeed very flat molecules and bind to the androgen receptor very firmly without any prior metabolic conversion. Other studies with methylated androgens also suggest that the receptor binds at multiple sites of an androgenic steroid simultaneously, as if the steroid molecule were being enveloped. This is in marked contrast to steroid-metabolizing enzymes or blood steroid-binding proteins, which generally recognize only a portion of the steroid molecule.

The localization of steroid-binding sites well inside the receptor proteins may be responsible for the very high affinity constants for the receptor binding of steroids, the extremely slow rates of association and especially dissociation of steroids from the receptor proteins (many hours) at low temperatures, acceleration of the rates of exchange of unbound steroids with bound steroids by freezing and thawing, and the inability of ethanol (30%) and detergent (2% Triton X-100 or deoxycholate) to free the steroid from the receptors in the cold (5).

The concept of steroid 'enveloping' suggests that the receptor protein and not the specific functional

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groups on steroids, participates in the key events leading to the hormone action. Additional support for this view of steroid 'enveloping' was obtained in our recent study on anti-steroid antibodies (14,15). Various antibodies against DHT and testoterone were found to be effective in removing steroid bound to nonreceptor proteins of blood (including human blood) and prostate, but were not capable of removing DHT bound to the receptor. This property is apparently a general one: we have also found that the antibodies for progesterone, estradiol, and dexamethasone can remove these steroids even when they are bound to blood globulins (Ka: $10^8 M^{-1}$) and non-receptor protein of their target tissues but can not remove steroids bound to their own receptors.

Based on these observations, we have devised a simple assay method for the qualitative and quantitative characterization of the steroid-receptor complexes (14,15). In this assay, the sample solution containing [^{3}H]-steroid-receptor complexes is mixed with antisteroid antibody that has the capacity to bind essentially all of the [^{3}H]-steroid present. The mixture is made to 0.4 M with respect to KCl and analyzed by gradient centrifugation under the conditions of the assay. The [^{3}H]-steroid molecules, in the free form or attached to non-receptor proteins, are bound by the antibody and sediment at 8 S, whereas the receptor-bound ^{3}H-steroid remains at the 3–4 S region and can be measured. The assay method can be simplified considerably by use of the antibody coupled to solid phases such as Sepharose. In this case, the assay mixture is simply centrifuged in a clinical centrifuge to sediment the steroid molecules that are not attached to the receptor originally but are now bound to the insolubilized antibody. The receptor content can, therefore, be determined directly from the radioactivity of the supernatant (Fig. 3).

By using this method, we have been able to show that the cell nuclei and cytoplasm of normal, hyperplastic, and cancerous human prostate contain DHT-receptor complexes that sediment at about 3 S in media with 0.4 M KCl.

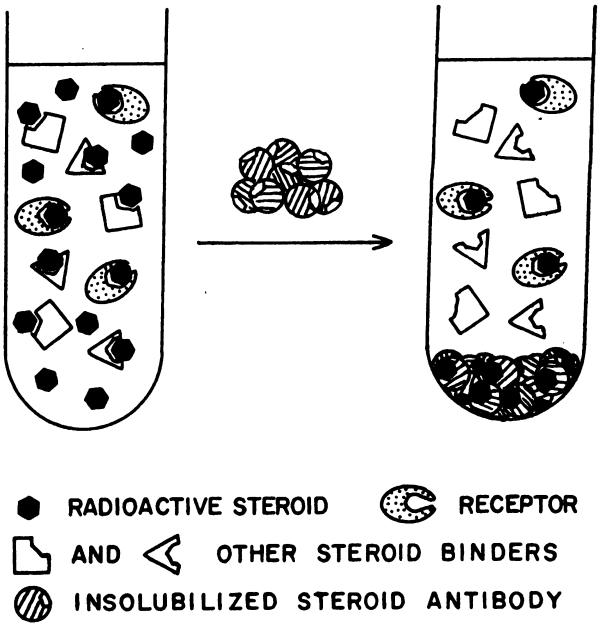

FIGURE 3. Diagram illustrating a new method for the characterization of ^{3}H-steroid-receptor complex by use of an anti-steroid antibody attached to a solid phase. The radioactive steroid, either free or bound to non-receptor proteins, can be removed from the solution (left tube) by precipitation with the insolubilized antibody (right tube). The ^{3}H-steroid that binds tightly to the hydrophobic pocket of the receptor is not affected and, therefore, can be estimated from the soluble fraction directly. [From Castaneda and Liao (14), by courtesy of Marcel Dekker, Inc.]

Antiandrogen and Receptor

Antiandrogens may act by preventing androgen synthesis or by altering the protein binding or peripheral metabolism of the circulating androgens (16). Studies in recent years have also suggested that some of these antiandrogens can act directly on the target tissues to antagonize the production, metabolism, or receptor binding of androgens (Fig. 1).

Some of the powerful antiandrogens appear to act by inhibiting receptor binding and nuclear retention of DHT. The earliest observation of this type was carried out with cyproterone and its 17α-acetate. These antagonists, injected into rats or added to prostate minces, can suppress the tissue uptake and nuclear retention of DHT (1,17). They can also inhibit the formation of receptor binding of DHT in cell-free soluble systems (5). Among other antagonists that appear to inhibit receptor binding of DHT in rat ventral prostate are SK and F 7690 (17α-methy-nortestosterone) (18), R-2956 (17β-hydroxy-2α, 2β, 17α-trimethyl-8α-estra-4,9,11-triene-3-one) (19), and BOMT (6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstane-3-one (20).

The effect of a nonsteroidal antiandrogen, flutamide (α-α-α-trifluoro-2-methyl-4′-nitro-m-propiono-toluidide or SCH 13521), can also suppress in vivo as well as in vitro, the receptor binding and nuclear retention of DHT (21,22). In vivo, flutamide may be hydroxylated to a more active form, however.

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These antiandrogens appear to act by their competitive binding on the androgen binding sites. However, the possibility that they may alter the conformation or subunit arrangement of the receptor complexes has not been explored. It is interesting to note that some antiglucocorticoids, such as cortexolone in rat thymocytes (23) and progesterone in hepatoma cells (24), can bind to the cytosol glucocorticoid receptors but that the complexes formed are not retained by the nuclei of these target cells. The antagonist-receptor complexes appear to be structurally incompatible with the nuclear acceptor sites. Such a study has not been made with antiandrogens. Theoretically, an antagonist-receptor complex can also bind to the nuclear site and thus prevent normal binding and functioning of the hormone-receptor complex at the nuclear acceptor sites.

Estradiol or diethylstilbestrol can also inhibit the receptor binding and nuclear retention of androgen in rat ventral prostate to some extent (1,25). In addition, they have been reported to inhibit the reduction of testosterone to DHT by the membrane-bound NADPH-dependent reductase (26). Progesterone and many weak androgens that are substrates for the reductase can also inhibit, by substrate competition, the generation of DHT from testosterone (27). In the rat ventral prostate, there are estrogen- and progestin-binding proteins that can be distinguished from the DHT receptor (5,28, 29). Whether estrogens and progesterone indeed act as antiandrogens by metabolic interference or protein binding is not clear.

Androgen Receptor and Gene Expression

In 1962, it was found that the prostate ribosomes isolated from androgen-injected rats have higher protein synthesizing activity than those from control castrated animals (30). This effect was attributed to the androgen-induced increase in the mRNA content of the prostate ribosomes and nuclei (31) and was observed many hours after androgen administration (Fig. 4). In 1965, we reported that the RNA-polymerase activity in prostate cell nuclei can be enhanced within 1 hour after castrated rats are given androgen (32). This early effect on RNA synthesis was found, by biochemical (33–35) and autoradiographic (36) studies, to occur most predominently at nucleolar chromatin regions that synthesize rRNA precursors. Other investigators have confirmed these early androgen effects (39,40) and shown that the effects are antagonized by antiandrogens (37,38).

Since the DHT-receptor complex can be retained

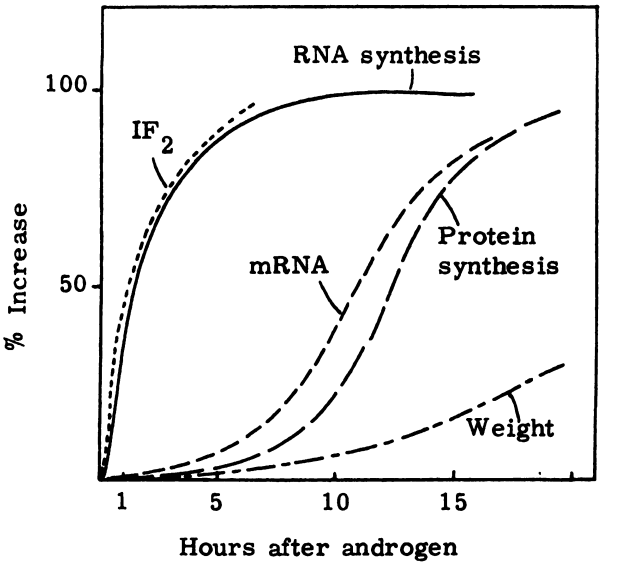

FIGURE 4. Early effects of androgen on the ventral prostate of castrated adult rats. Biochemical parameters shown are the activity of the initiation factors (IF_2) in the rat cytosol, RNA synthesizing activity (mostly nucleolar RNA synthesis) of isolated cell nuclei, mRNA activity of the nuclear and ribosome-bound RNA, protein synthesizing activity of the isolated ribosomes, and the fresh weight of the ventral prostate. [See text for sources of the data.]

by the prostate cell nuclei where the rapid effect of androgen can be observed, a primary site of androgen action appears to be the nuclear chromatin. Although a definite proof for this view is not yet available, Davis and Griffith have reported that the RNA synthesizing activity of isolated prostate cell nuclei can be enhanced by the addition of a relatively low concentration of DHT, providing that a prostate cytosol fraction, presumably containing an active form of the androgen receptor, is also present (41).

The possibility that a steroid hormone and its receptor may play a role in post-transcriptional processes has been considered by several workers (1–3,42). We initially studied the capability of the DHT-receptor complex of the rat ventral prostate (42) and the 17β-estradiol-receptor complex of rat and calf uterus (42,43) to interact with the ribonucleoprotein (RNP) particles in the cell nuclei and in the cytoplasm. These studies have shown that the steroid-receptor complex can bind to a small group of nuclear RNP particles and to certain ribosomal subunit particles (40 S and 60 S), but not to the 80 S monosomes or polysomes. The precise requirement for such specificity is far from clear. It is interesting to note, however, that the steroid-receptor-associated 40–50 S RNP particles appear to form in

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vivo and that aurin tricarboxylic acid (ATA), which can partially dissociate the 40 S ribosomal subunit particle and inhibit the initiation of protein synthesis, can prevent the steroid-receptor complexes from binding to the RNP particles.

We have, furthermore, reinvestigated the early effect of androgen on the factors that may influence protein synthesis in the rat ventral prostate (44). Special attention has been given to the factors that affect the initiation process required for protein synthesis. Detailed examination of the initiation process has indicated that it is essentially identical to the process proposed for bacterial and other eukaryotic systems. The methionylated $tRNA_f$ first binds to an initiation factor (IF_2 protein, according to the terminology used in the bacterial system) in the presence of GTP. The [^{35}S] met-tRNA-IF_2 complex can then bind to a small ribosomal subunit particle to form the [40 S-methionyl-$tRNA_f$] initiation complex. These radioactive complexes can be retained on Millipore filters and assayed.

As shown in Fig. 5, formation of the initiation complex is stimulated more by the cytosol from normal rats or from rats which have been castrated and androgen-injected than by that from the control castrates. The initiation factor activity decreases within hours after castration. This decrease is reversed within 1 hour after an intraperitoneal injection of a milligram quantity of DHT. If intravenous injection is used, a microgram quantity of DHT can have a significant effect within 10 minutes. We have consistently observed a lower activity at 30 minutes than at 10 minutes; this suggests that a continuous supply of androgen may be needed for maintenance of the cytosol activity.

At this time, it is premature to eliminate completely the possibility that the synthesis of a specific species of RNA may be required for the androgen effect. We have found, however, that neither the removal of RNA from the cytosol nor the injection of Actinomycin D (200 μg to 500 μg per rat) eliminates the androgen effect (Table I). The synthesis of a new protein does not appear to be necessary, since cycloheximide also did not eliminate the effect.

Table II shows that the antiandrogen, cyproterone acetate, which inhibits the receptor binding of DHT, can suppress the cytosol IF_2 activity. This finding and the aformentioned observation of the binding of the receptor to the ribosomal subunit particles indicate the possibility that the receptor binding of androgen may have a regulatory role in the protein-synthesizing mechanism within the target cells. Since proteins in our partially purified

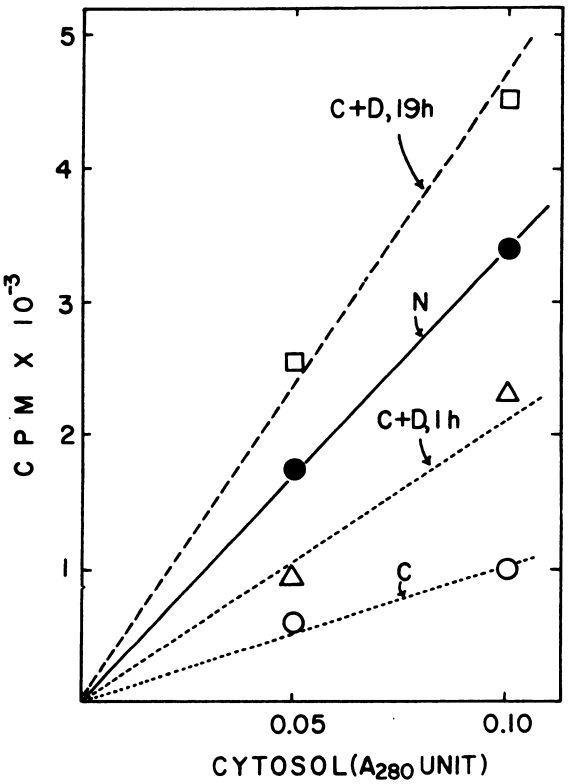

FIGURE 5. Effect of an intraperitoneal injection of DHT on the cytosol initiation factors. The prostate cytosol fractions were obtained from normal rats (●) castrated 19 hours previously (o), and from castrated rats which were injected with 2.5 mg DHT immediately after castration (□) or 1 hour before being killed (Δ). The binding of [^{35}S]methionyl-$tRNA_f$ to the cytosol initiation factor was assayed in the presence of prostate ribosomes. Millipore filters were used to trap the radioactive initiation complex formed. The effect can be demonstrated within minutes after the rats receive the androgen. [From Liang and Liao (44), by courtesy of the National Academy of Sciences.]

DHT-receptor preparations exhibit some [^{35}S]met-tRNA binding activity, we have been exploring the possibility that the androgen-receptor complex may contain the initiation factor activity. Preliminary experiments based on gradient centrifugation, however, showed that the major part of the [^{35}S]met-$tRNA_f$ binding activity can be separated from the DHT-binding receptor protein.

Summary

In rat ventral prostate, 5α-dihydrotestosterone (DHT) appears to function as an androgen by first binding to a specific intracellular receptor in the cell cytoplasm. The steroid-receptor complex is then translocated to a nuclear (chromatin) site by a temperature-dependent process. The sedimenta-

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TABLE I—Effect in Vivo of 5α-Dihydrotestosterone and Actinomycin D on the IF Activity of Prostate Cytosol [a]

Source of cytosol	[^{35}S] met-tRNA$_f$ binding (cpm)	
	Control	+ Act. D
Castrates	24,120	23,160
Castrates + DHT	33,670	34,580

[a] Experimental conditions were the same as in Fig. 5. Rats had been castrated 19 hours previously. Actinomycin D (200 μg per rat) and DHT (2.5 mg per rat) were injected intraperitoneally 2.5 and 2.0 hours before the rats were killed. Each experimental tube contained 50 μg of cytosol protein of the ventral prostate. Other experiments also showed that Actinomycin D was not able to reduce the 10-minute effect of DHT.

TABLE II—Effect in Vivo of 5α-Dihydrotestosterone and Cyproterone Acetate on the IF Activity of Prostate Cytosol [a]

Source of cytosol	[^{35}S] met-tRNA$_f$ binding (cpm)		
		+ Cyproterone acetate	
	Control	5 mg/rat	10 mg/rat
Castrates	5,936	6,905	3,418
Castrates + DHT	12,067	6,587	4,278

[a] Experimental conditions were the same as in Table I. Cyproterone acetate (5 or 10 mg per rat) and DHT (2.5 mg per rat) were injected simultaneously 2 hours before the rats were killed.

tion pattern of the complex varies drastically when the pH of the medium changes from pH 6.4 to 9.5, and with certain divalent metal ions. For example, Zn^{++} appears to interact and alter the receptor proteins in such a way that its affinity toward DHT is reduced.

Other potent androgens besides DHT appear to bind to the hydrophobic cavity of the receptor protein as if the steroid were enclosed on all sides. The bulkiness and flatness of the steroid molecule seems to play a far more important role in receptor binding and androgenicity than the detailed electronic structure of the steroid nucleus. The concept of steroid enveloping suggests that the receptor protein and not the specific functional groups on steroids plays the key role in triggering androgen action.

Antisteroid antibody can remove the steroids attached to blood globulins and non-receptor proteins of their target tissues but not the steroids bound to their own receptors. Based on this difference, we have devised a simple assay method for qualitative and quantitative characterization of the steroid-receptor complexes. Using this technique, we have been able to show that the cell nuclei and cytoplasm of human prostate contain DHT-receptor complexes that sediment as 3 S in media with 0.4 M KCl.

In vivo DHT can enhance the activity of the cytosol initiation factor that is required in protein synthesis. Such stimulation can be observed within 10 minutes after the androgen is administered to castrated rats. The androgen effect appears to be on the protein (IF_2) that binds the initiator methionyl-tRNA$_f$. The effect is not abolished by Actinomycin D or cycloheximide. Antiandrogen, such as cyproterone, which inhibits the binding of DHT to the receptor, abolishes the androgen effect on initiation factor activity. The androgen-receptor complex, therefore, appears to have an immediate effect on the cytoplasmic protein synthesizing machinery of the target cells.

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DISCUSSION

S. G. KORENMAN: Are you really saying that there is a direct translational effect of the androgen in the prostate?

S. LIAO: I still believe that androgens (and receptors) can regulate RNA synthesis in the cell nuclei of target tissues, but I wouldn't say that all androgen effects are dependent only on nuclear events. There is no doubt that the prostate of animals castrated for long periods are deficient in all types of RNA and require increased rates of RNA synthesis for growth and function. In such a system many long term (> 1 hr) effects of androgens are dependent on RNA synthesis, but the possible direct action of androgens at other cellular sites should not be overlooked. In target cells steroid hormones may exert coordinated control on both transcription and translation.

N. BRUCHOVSKY: You showed a decline in cytosol receptor concentration following castration. Have you looked at the stimulation of RNA synthesis at a time when receptor concentration in the cytoplasm is very low or absent to see whether an effect on RNA synthesis can be demonstrated in the absence of a receptor?

LIAO: We have not tried to correlate receptor concentration and the stimulation of RNA synthesis. In general, a more rapid androgen effect on RNA synthesis is observed with rats castrated 2 to 3 days than with rats castrated more than a week. However, the percent increase may be larger with the latter group of animals because of the lower control values. The rapidity and the extent of increase may be controlled not only by the receptor content, but by other cellular conditions such as RNA polymerase proteins.

S. A. SHAIN: We'd like to confirm your observation about the half-life of the receptor in castrated animals. Last year at the Endocrine meetings we reported some preliminary data which indicated that the dorsolateral prostate of the rat did not contain demonstrable cytoplasmic receptors; yet, high affinity binding could be demonstrated in dorsolateral prostate nuclei. There are a valid criticism of these initial experiments in that we did use relatively low specific activity 5α-dihydrotestosterone, about 50 Ci/mmol. All the experiments have been repeated employing 5-DHT at 160 Ci/mmol, and the results were identical. All of us appreciate the fact that the dorsolateral prostate shows androgen dependence very similar to that of the ventral prostate. If there is no demonstrable cytoplasmic receptor in the dorsolateral prostate and yet there is a nuclear receptor that shows steroid specificity identical to that demonstrable for the ventral prostate, one might conclude that all the effects of androgen are not mediated through the action of cytoplasmic receptor alone. Furthermore, if one considers the data of Tomkins for the HTC system, castration should result in the appearance of nuclear receptor in the cytoplasm. However, cytoplasmic receptors are not demonstrable in the dorsolateral prostate following castration. We do not think that the lack of cytoplasmic receptors is a consequence of unequal distribution between nucleus and cytoplasm.

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STUDIES ON THE ROLE OF MACROMOLECULAR SYNTHESIS IN THE GROWTH OF THE PROSTATE

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Normal and abnormal growth of the prostate gland is composed of two processes which include hyperplasia and hypertrophy. Hyperplasia may be defined as an increase in the absolute cell number and can be monitored by determination of the total cells, nuclei, or total DNA content of the gland. Hypertrophy is defined as an increase in the cellular size of the constituent cells. This may be monitored by the determination of an increase in the cytoplasmic components of the individual cells and can be reflected by the RNA to DNA ratio of the gland. Both of these processes are depicted in Fig. 1, where following castration there is both a decrease in the total number of cells in the gland, as well as a marked decrease in the cellular volumes. This involution is usually reflected in the epithelium as a transition from columnar to cuboidal form of the cells. In castrates, the exogenous administration of steroid hormones can modify these growth parameters through restoration of hypertrophy, hyperplasia, or a combination of both. These changes may be monitored by biochemical analysis of the total DNA and RNA content of the gland as well as the synthetic processes which produce a net accumulation of these nucleic acids. In addition, quantitative histological techniques, morphometrics, reflect a profile of response occurring in the various types of cellular components including the stromal and epithelial elements. For a more complete analysis of the growth properties of the prostate, information is required on the nucleic acid content as well as the quantitative determination of the cellular elements through morphometric determinations. The combination of these two techniques provides a new method in the analysis of prostate growth which we termed *biomorphometrics*.

I. Nucleic Acid Synthesis

It is now well established that androgens produce marked alterations in nucleic acid metabolism in sex accessory tissue. Williams-Ashman and Reddi (1) have presented a comprehensive review on the effects of androgens on tissue growth and function. In addition, Epifanova (2) has reviewed the specific effects of many types of hormones on the cell cycles and kinetics of growth in mammalian tissues. Lesser and Bruchovsky (3) have determined the effects

Supported by Public Health Service Grant CA-15416 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

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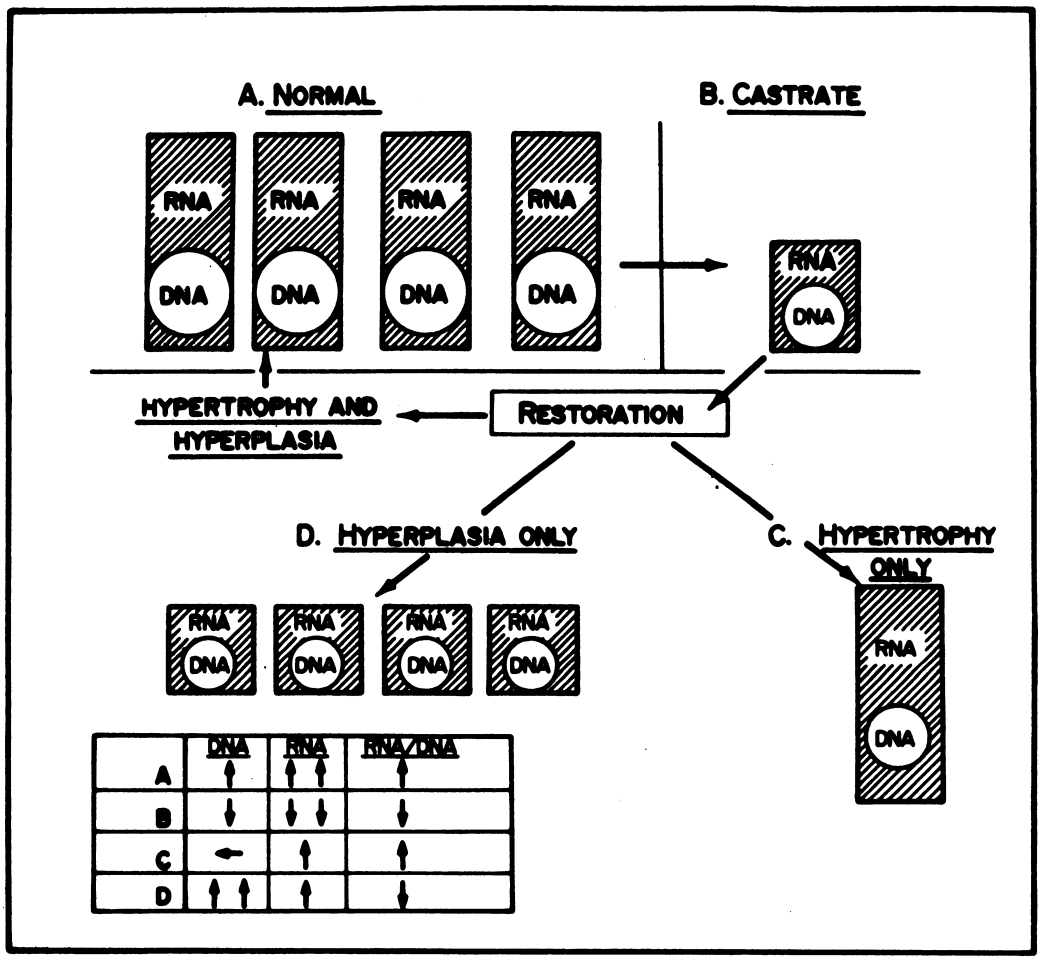

FIGURE 1.

of 5α-dihydrotestosterone on the kinetics of cell proliferation in the rat prostate.

Many investigators have concentrated on the biochemical events associated with the androgen-induced alterations of RNA metabolism. This is primarily due to the fact that this represents an early event of hormone action on both male and female sex accessory tissues (1,4). In contrast, the androgen-induced stimulation of DNA synthesis is a relatively late event of hormone action and has received only minimal attention.

A. RNA Metabolism

Tata (5) has reviewed the many examples where hormones are known to have elicited a stimulation of nuclear RNA synthesis. Relevant to this is the work of Williams-Ashman and his associates who in 1962 reported the classic observation that the administration of androgens to castrate rats greatly increased the incorporation of cytidine triphosphate (CTP) into RNA by an aggregate enzyme preparation isolated from nuclei in the ventral prostate gland (6). Subsequently, several investigators observed that androgens stimulate RNA synthesis in the seminal vesicles (7,8) and ventral prostate (9). Liao and his associates demonstrated that the primary stimulation was in the synthesis of ribosomal RNA [(10,11) and review (12)]. The activation of RNA synthesis can involve several processes including: (a) activation of the chromatin template which has been implicated in several studies (10, 13); (b) availability of the nucleotide precursors for RNA; (c) the possible involvement of cytoplasmic or initiating factors as discussed by Harris (14) which are required for other mammalian systems; and (d) the activation or increase in levels of the RNA polymerase enzymes.

Many of the early studies on the hormonal control of enzymatic synthesis of RNA will require addi-

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tional evaluation because it is now possible to solubilize and resolve mammalian nuclear RNA polymerase activity into several components with different sub-nuclear localization properties. Early attempts have been made to solubilize prostatic RNA polymerase, but these studies were limited because only a partial solubilization of the total enzymatic activity was realized (15). The more complete and reproducible solubilization of total nuclear RNA polymerase has been achieved by Roader and Rudder (16,17), and distinct enzymes have been identified in the nucleolar and extranucleolar fractions of the nuclei from rat liver and sea urchin oocytes. A similar approach has now been applied to prostatic RNA polymerase by Liao, et al. (18) and Mainwaring, et al. (19). The total prostatic nuclear RNA polymerase activity has been separated into two components, one associated with nucleolus and the other extranucleolar. Two hours after androgenic hormone administration *in vivo* a particular pronounced stimulation was observed in the activity of the nucleolar enzyme; this precedes activation of the nucleolar template (20). This would tend to support the observations of Liao that stimulation of ribosomal RNA synthesis is one of the early effects of androgens since the nucleolus is the principal site of ribosomal snythesis in mammalian cells (21,18). It is unclear at present whether the androgen induced increase in nucleolar RNA polymerase activity is associated with (a) enhanced synthesis of the enzyme; (b) stabilization of the enzyme to inactivation or degradation; (c) removal of inhibitor or regulatory factors; or (d) activation of the enzyme directly by the steroid or hormone-receptor complex. With regard to the latter point, Bashirelahi, et al. (22) have reported a direct effect of dihydrotestosterone *in vitro* on the stimulation of RNA synthesis in isolated prostatic nuclei; however, Mainwaring, et al. (19) were unable to confirm these findings. The interactions of steroid with chromatin might require the intervention of the hormone-receptor (20). Hough, et al. (23) have demonstrated that the activity of the uterine nuclear RNA polymerase can be stimulated *in vitro* in the presence of the 17β-estradiol-receptor complexes. In any experiment designed to test the *in vitro* effects of hormone receptors on template properties of chromatin, one should be cautioned to consider contaminating proteins in the hormone receptor fraction. For example, contaminating DNase can nick the DNA of the chromatin, thus providing additional template activity; or, proteolytic activity in the hormone receptor fraction could alter the nucleoproteins and release DNA template restrictions.

Several other observations have been reported in relation to estrogen action on the uterus which might also be tested for a similar effect in androgen sensitivity tissues. For example, in the uterus the estrogen induction of RNA synthesis depends on the preliminary synthesis of a specific protein fraction (24,25). In addition, new techniques are now available to determine the specific synthesis of messenger RNA's, and it would be of paramount importance to resolve the temporal requirements in the synthesis of various types of nuclear RNAs which follow androgen stimulation.

Dr. Kahlil Ahmed and his associates (26,27,28,29) have made several important reports concerning the early action of androgens on the prostatic nuclei which include the ability of ATP-γ-^{32}p to phosphorylate nuclear proteins. The role of this nuclear phosphorylation reaction has not been elucidated in the control of macromolecular synthesis in the prostate but could be of extreme importance. The possible involvement of cyclic AMP in this phosphorylation reaction has been eliminated by the studies of Wilson and Ahmed (29). In this regard, the absence of effects of cyclic AMP on cell proliferation in the rat prostate has been established by the studies of Lesser and Bruchovsky (3). This should not be interpreted that cyclic AMP has no effect whatsoever on the prostate because Singhal, et al. (30) have provided evidence for the involvement of cyclic AMP as a mediator for androgenic stimulation of certain prostatic enzymes. Therefore, it might be concluded that cyclic AMP is involved in the stimulation of certain enzymes but not in the stimulation of cellular proliferation.

Recently, Goldberg and his associates have drawn attention to the importance of cyclic-GMP as a mediator for cell proliferation in many tissues including the estrogen stimulated uterus (21,32,33). Extensive studies have not been carried out on the effects of cyclic GMP on cell proliferation and nucleic acid snythesis in the prostate although preliminary studies have been reported by Thomas and his colleagues (34). Unfortunately, cyclic GMP levels usually are an order of magnitude lower than cyclic AMP in tissues, and precise and accurate assays are being developed in many laboratories to delineate further the analytical approach to this problem.

It is now apparent that RNA synthesis, both ribosomal and messenger RNA, probably is an early event in hormone response to sex accessory tissues. Recently, Liang and Liao (35) have reported on an initiation factor appearing in the prostatic cytosol 10 minutes after the intravenous injection of dihydrotestosterone into castrate rats, and

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this factor stimulates the binding of [^{35}S]-methionyl-tRNA$_f$ which binds to the 60S ribosomal subunit isolated from prostatic tissue. They conclude that this protein initiation factor occurs almost immediately after an androgen enters the target cell. It is unknown at the present whether this induction depends on the synthesis of nuclear RNA; however, actinomycin D was not able to block this effect. The identity of the initiator has not been established, and it is unknown whether it is directly related to the hormone receptors in the cell.

B. DNA Metabolism

The effects of androgen administration on the proliferative phase of androgen-induced growth in sex accessory tissues was first observed in 1942 as an increase in the mitotic activity by histological examination as reported by Burkhard (36). Subsequently, there were many studies on the effects of castration and androgen replacement on the nucleic acid content of these tissues (37,38,39,40, 41,42). With the development of radiolabeling techniques it was possible to ascertain the rate of incorporation of several types of precursors into DNA and determine the rate of DNA synthesis both in vivo and in vitro (43,44,45,46,47,48,3).

In the castrated rat, the androgen-induced growth of the ventral prostate gland is marked by two distinct and characteristic processes. One component is an increase in the hypertrophic element of the cell as measured by an increase in RNA and protein synthesis. These events occur very rapidly, and significant increases are noted within a few hours following initiation of hormone treatment. In contrast, the hyperplastic growth, which is marked by an increase in DNA synthesis and subsequent cell division, is a late event following androgen treatment; it occurs after a lag period of about 24–48 hours and attains a maximum at 3 days (45,49). This onset in the rate of DNA synthesis is accompanied by a massive but transitory increase in the activity of a "replicative" type of prostatic DNA polymerase which preferentially utilizes single stranded DNA template and requires all four deoxyribonucleotide triphosphates. The activity of this enzyme occurs largely in the cytoplasmic rather than the nuclear fraction of prostatic homogenates prepared in 2.2M sucrose. The ventral prostate of the normal intact animal, as well as castrates, had a very low DNA polymerase activity. The increase in DNA polymerase activity accompanies the increase in the rate of DNA synthesis which was determined both in vivo and in vitro using several labeled precursors of DNA ([^{3}H]-thymidine, [^{14}C]-formate, and [^{14}C]-glycine). The increase in the activity of DNA polymerase declined with time even though the animals continued to receive the hormones, and this was accompanied by a cessation of the hyperplastic element of growth in the gland (Fig. 2). At present, it has not been determined whether the increase in DNA polymerase activity is a reflection of the DNA repair enzyme or truly a replicative type of reaction. In addition, Shimazaki, et al. (50) have reported on the levels of DNA polymerase in several types of urogenital tumors including prostatic cancer and benign prostatic hyperplasia.

It has been reported that androgens were only required in the early phases of growth during the G_1 phase of the cell cycle (51). For example, using the antiandrogen, cyproterone acetate, androgens could be displaced from the nucleus in the late G_1 period, and this would not diminish the observed rate of DNA synthesis. Carter et al (51) concluded that the presence of androgens in the G_1 period initiated a sequence of events which later resulted in DNA synthesis, and this cascade effect could not be blocked by removing the androgen just before the onset of the S phase. Subsequent studies by Chung and Coffey (52,53) determined that alterations occurred in the prostate nuclear membrances which just preceded the onset of DNA synthesis. Whether these changes were concurrent or causal was not established. These latter studies have been recently reviewed (54).

More recently, Sufrin and Coffey (55) have studied the ability of various antiandrogens to block the induction of DNA synthesis in the rat ventral prostate. This has provided a precise and rapid model for determining the antiandrogenic potency of several compounds which are currently in preliminary clinical trials in patients with benign prostatic hypertrophy and adenocarcinoma. In addition, Sloan, et al. (56) have adapted this model to study the effects of non-hormonal drugs such as particular cancer chemotherapeutic agents in blocking DNA synthesis in the rat ventral prostate following androgen induction.

II. Biomorphometrics

The above studies on the accumulation and synthesis of DNA and RNA in the rat ventral prostate in different phases of growth shed important insight into the various growth parameters de-

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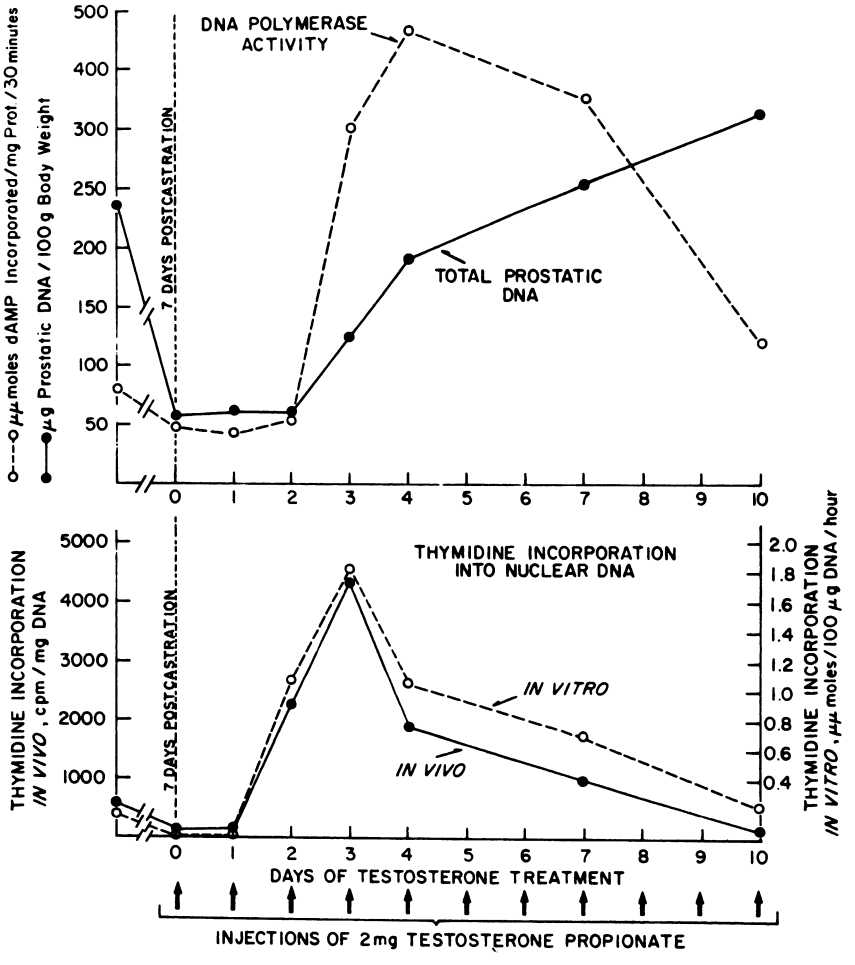

FIGURE 2.

picted in Figure 1. However, they do not reflect variations in the response of the different components and specific cell types of the ventral prostate. To approach this problem, we have developed a combination of these chemical determinations with the standard morphometric procedures as described by Weibel (57). Morphometric analysis involves super-imposing a point-grid system over a histological section and quantitating by statistical methods the specific cell types and components of the prostate. This includes quantitative determination of epithelial cells, stromal cells, and their nuclei and cytoplasmic components. In addition, the percent of acinar volume, epithelial volume, and stromal volume can be determined in a precise manner. When the total volume of the gland is known the individual component volumes can be calculated. In addition, knowing the percentages of the various types of nuclei combined with the knowledge of the total DNA in the gland and the nuclear DNA content, enables one to calculate the histological and cellular profile of the gland in a quantitative manner. The combination of the chemical determinations of macromolecular content of the prostate (DNA and RNA) and the morphometric determination of specific cell distribution has provided an important new approach to understanding the growth of tissue which we have termed *biomorphometrics*.

Using this new technique of biomorphometry, we have determined the effects of various androgens and an estrogen on the growth of the rat ventral prostate in castrate adult animals. These animals were castrated for seven days and then received daily injection of 5 milligrams per day of the indicated androgen or where indicated 1 milligram per day of 17β-estradiol. The injections were made subcutaneously in 0.2 ml. of propylene glycol, and each animal was injected daily for 10 days. At the end of the experiment, the ventral prostate was removed, and the DNA and RNA contents were determined by methods previously reported (45). Histological sections were removed and stained with hematoxylin and eosin. The cell numbers and distribution were determined with a calibrated occular grid. For each specimen, 400 nuclei were counted, and for the

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morphometric analysis of the individual component volume ratios 2000 point determinations were recorded. Table 1 indicates the biochemical determination of the RNA and DNA content of the gland as well as the wet weight. Table 2 contains the biomorphometric analysis of the ventral prostate following hormonal manipulation. It is apparent that the normal gland contains over 4 epithelial cells for each stromal cell, and that this ratio is reduced 10 fold by castration so that the stromal cells exceed the epithelial cells by a factor of 2. There is an 80% reduction in the total number of cells in the prostate following castration. This overall reduction is composed of a 93% decrease in epithelial cells while the stromal cells decrease by only 22%. It appears that the epithelial cells are more sensitive to androgen deprivation. If the castrated animals are treated with testosterone (5 mg/day) for 10 days, the epithelial to stromal ratio is only partially restored. While the epithelial cells return to 67% of the normal value, the stromal cells increased 18% above that present in the normal gland. These effects are magnified with dihydrotestosterone where the epithelial cells restored to normal values while the stromal cells almost double in number. Similar effects were also observed when 5α-androstane-3α, 17βdiol was administered. In contrast, 5α-androstane-3β, 17βdiol has no stimulatory effect on the stromal cells, and there is only a slight stimulation of epithelial cell number. This is similar to what is observed with estrogen treatment of the castrate animals. Tables 1 and 2 have enabled us to determine in a quantitative manner the hyperplastic component of prostatic growth.

Administration of exogenous steroids not only induce hyperplasia, but also cellular hypertrophy. Numerous attempts have been made to quantify the hypertrophic response to a growth stimulus. This has been expressed as: 1) epithelial height, 2) nuclear to cytoplasmic area, and 3) RNA to DNA ratio. The limitations of all these methods are obvious. Measuring the epithelial cell height is express-

TABLE I—Effect of Steroid Hormones on Wet Weight, DNA, and RNA Content of Ventral Prostate

	Condition	Body Weight (g)	Gland Wet Weight Per 100g B.W.	Total DNA (μg)	Total RNA	RNA/DNA
	5 mg/day × 10					
Normal	----	399.7 ± 5.9	117.5 ± 7.0	845.2 ± 45	2,400 ± 147	2.81
Castrate	----	347.0 ± 7.2	8.7 ± 0.7	171.4 ± 19	45 ± 15.4	0.27
Castrate	Testosterone	378.8 ± 7.7	85.8 ± 12.0	641.9 ± 74	1,405 ± 195	2.18
Castrate	Dihydrotestosterone	386.0 ± 12.3	111.3 ± 9.1	1,024.4 ± 228	1,878 ± 361	1.78
Castrate	5α-androstane-3α, 17β diol	387.0 ± 6.4	97.65 ± 6.2	719.3 ± 110	1,984 ± 132	2.83
Castrate	5α-androstane-3β, 17β diol	371.6 ± 16.9	32.8 ± 1.0	283.3 ± 60	654 ± 127.3	2.31
Castrate	(1 mg/day × 10) 17β-estradiol	259.5 ± 16.0	17.6 ± 1.1	263.9 ± 20.3	75.4 ± 31.9	0.28

TABLE II—Effect of Steroid Hormones on Cellular Content in the Rat Ventral Prostate

	Condition	Total Cells		Stromal Cells		Epithelial Cells		Ratio Cells Epithelial/ Stromal
		Total Number	Relative Number	Total Number	Relative Number	Total Number	Relative Stromal	
	5 mg/day × 10	(10^7)		(10^7)		(10^7)		
Normal	----	6.85 ± 0.4	100 ± 5	1.23 ± 0.1	100 ± 5	5.62 ± 0.3	100 ± 5	4.54
Castrate	----	1.39 ± 0.2	20 ± 2	0.97 ± 0.1	78 ± 9	0.42 ± 0.1	7 ± 1	0.43
Castrate	Testosterone	5.21 ± 0.6	76 ± 9	1.46 ± 0.2	118 ± 13	3.75 ± 0.4	67 ± 8	2.57
Castrate	Dihydrotestosterone	8.32 ± 1.8	121 ± 27	2.45 ± 0.5	198 ± 44	5.87 ± 1.3	104 ± 29	2.40
Castrate	5α-androstane-3α, 17β diol	5.84 ± 0.9	85 ± 13	2.06 ± 0.3	166 ± 25	3.78 ± 0.6	67 ± 10	1.84
Castrate	5α-androstane-3β, 17β diol	2.30 ± 0.5	33 ± 7	0.95 ± 0.2	77 ± 16	1.35 ± 0.3	24 ± 5	1.42
Castrate	(1 mg/day × 10) 17β-estradiol	2.13 ± 0.2	31 ± 2	0.99 ± 0.1	81 ± 6	1.14 ± 0.1	20 ± 2	1.14

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ing but one dimension of cellular growth which is influenced not only by the size of the individual cell but also may be influenced by exterior compression such as from secretion collected in a glandular acinus. The nuclear to cytoplasmic area ratio is unsatisfactory as the nuclear size might change along with the actual increase in the cytoplasmic fraction so that although a marked increase may be seen in both the nucleus and in the cytoplasmic area the ratio may remain constant. The RNA to DNA ratio is a satisfactory measure of hypertrophy in the whole gland but does not express the individual components of the gland such as the stroma and epithelial elements.

Previous authors have advocated the quantification of hypertrophy as the cytoplasmic volume per nucleus, and this type of measurement has been made in the gut wall and muscular components of the urinary tract (58,59). We have chosen to apply morphometric techniques described by Cussen (59) to the sex accessory tissue, and a summary of the results as obtained are presented in Table 3. It will be noted that the epithelium in the intact controls and androgen treated animals had a similar degree of hypertrophy. No significant difference was noted between testosterone, dihydrotestosterone, or the androstanediols. Castration, however, led to a 82% decrease in the cytoplasmic volume per cell. The estradiol treated group had a 72% decrease in the cytoplasmic volume per cell as compared to the intact control group. In the stroma, none of the androgen-treated groups were able to restore the stromal components to the same degree of hypertrophy as was seen in the intact control group during the ten day period of treatment. Dihydrotestosterone produced a slightly higher degree of hypertrophy in the stroma that testosterone, 5α-androstane-3α,17βdiol, and 5α-androstane-3β, 17βdiol, but this difference was not significant. Castration produced a 36% decrease in the cytoplasmic volume per stromal cell; this decrease was found to be significant.

We are aware that there are problems in the techniques described above. In the first place, variations in the nuclear size may influence the actual counts of nuclei per volume, such as have been described by Elias and Hennig (60,61). Differences in the nuclear size were minimal in the tissues that we have studied, and we do not think this has significantly influenced our results. However, if this type of measurement is extended to other tissues, the possible influence of nuclear size should be kept in consideration. Secondly, these measurements were made on tissues which had been stimulated for a definite period of time, and it is possible that these differences may change after a long period of androgen stimulation; thus the maximal response to a steroid such as 5α-androstane-3β, 17diol may require a longer period of administration of the steriod than with another androgen such as dihydrotestosterone. However, bearing these limitations in mind, and others which have not been discussed, we feel that these techniques may have important applications in the study of the male sex accessory tissue and their response to androgen stimulation and deprivation.

In conclusion, biomorphometric techniques have provided new insight into the various facets of prostatic growth with particular emphasis on cell types in relation to hyperplasia and hypertrophy. It is apparent that various types of androgens and estrogens have remarkably different properties in their ability to stimulate the various cellular components of prostatic growth. These techniques are

TABLE III—Hypertrophy of the Epithelial and Stromal Elements of the Ventral Prostate

Experimental Conditions			Total Gland RNA/DNA	Epithelium			Stroma		
Castrate	Intact	Steroid		Cell Height	% of Intact	Cytoplasmic Volume Per Cell	% of Intact	Cytoplasmic Volume Per Cell	% of Intact
		(5 mg/day × 10)		(μ)		(μ³)		(μ³)	
–	+	----	2.81	26.3 ± 0.6	100	351.5 ± 18	100	495.6 ± 57	100
+	–	----	0.27	8.8 ± 0.4	33	62.0 ± 8	18	315.3 ± 22	64
+	–	Testosterone	2.18	28.8 ± 1	109	426.7 ± 8	121	380.9 ± 19	77
+	–	Dihydrotestosterone	1.78	26.8 ± 0.7	102	391.8 ± 44	111	420.1 ± 55	85
+	–	5α-androstane-3α, 17β diol	2.83	24.7 ± 0.5	94	406.6 ± 43	116	371.1 ± 36	75
+	–	5α-androstane-3β, 17β diol	2.31	20.4 ± 0.5	77.6	342.7 ± 30	98	331.1 ± 49	67
+	–	17β-estradiol	0.28	8.18 ± 0.3	31	99.0 ± 18	28	360.7 ± 36	73

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important because of the well known variation in abnormal growth in human prostatic tissue. For example, Franks has described five types of benign prostatic hyperplasia with regards to the histological appearance (62). To our knowledge, only one paper has appeared on the quantitative aspects of the morphometric determinations of human benign prostatic hyperplasia (63). Although they did not apply the more complete biomorphometrics to these procedures, it is apparent that these techniques will provide important insight into the various elements of growth resulting from benign prostatic hyperplasia. It is also important to establish these values in the development of the prostate at various ages in order to determine the specific cell types which either predispose or result in the early formation and development of the benign lesion. It is important to distinguish hypertrophic from hyperplastic elements in these growth processes.

Considerations

For many decades, extensive studies have been carried out on the structure and activity of steroid molecules in relation to their androgenic properties in inducing growth of sex accessory tissue. It is now apparent that a much higher resolution is required to elucidate fully their specific effects on various organs and on specific cell types within these glands. For example, it is now apparent that there can be a differential effect of the steroid molecule on various lobes of the prostate and on other sex accessory tissues. Therefore, in determining androgenicity, it is important to consider what cell type is used as the biological indicator. Furthermore, androgens appear to have markedly different effects at various ages. We and others have observed marked differences in whether androgenicity is determined by maintaining the size of the gland, by immediate administration of the hormone following castration, or in the restoration of a fully involuted prostate. Androgens have long been known to be important factors in utero in the development of the male reproductive system. More recently, it has become apparent that they may also mark the gland during the neonate period in the first five days following birth, and this can have subsequent effects on androgenic responses in later life. The full effects of many of these androgens on prostatic growth have not been determined for extensive time periods and in various dose levels or indeed in combination with estrogens and other androgen metabolites. In all of the above studies, it would appear that biomorphometric measurements would shed important new insight into the specific effects of pharmacological properties of these steroid compounds.

At present, there are only limited studies on the effects of steroid molecules on the specific stimulation of stromal components. Many of these studies have not been of a quantitative nature that would provide definitive analysis of these differential effects. In addition, the effect of the stromal elements on the growth of the epithelial cells has often been alluded to but has not been resolved. In the present review, we have attempted to review past concepts and present some new approaches which may be helpful in elucidating many of the above problems. It is apparent that such gross measurements as stromal cell numbers must now be broken down into the various components such as smooth muscle, fibroid elements, the connective tissues, and myoepithelial cells. It is anticipated that the availability of this information might provide a more complete understanding of the process of the development of benign prostatic hyperplasia and other abnormal growths of the prostate. In addition, the careful study of the aging process in various animal systems with particular reference to their hormone responsiveness may provide new model systems to study this disease process.

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DISCUSSION

J. D. Wilson: If antiandrogen is administered at the peak of the DNA synthesis, does it delay the fall in DNA synthesis?

D. S. Coffey: We have not done that. In other words, when it reaches the peak, give large amounts of antiandrogen and see if a turnoff mechanism is apparent. All we know is that it stops at that point. We have not looked at the mechanism of the turnoff.

P. Ofner: I should like to address myself to the problem of 3β-androstanediol (3β-diol) metabolism in the prostate, pertinent to the evaluation of its intrinsic androgenic potency. In our paper on the subject (Ofner *et al.*, Steroids *24:* 261, 1974) ^{14}C-3β-diol was actively converted by minced canine prostate and epididymis and human prostate adenoma to 7 (preponderant)- and 6-hydroxylated derivatives and minimally to 5α-dihydro-

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testosterone. We have since found that minced rat ventral prostate is more active than the canine and human tissue in hydroxylating the 3β-diol. In contrast, minced rat ventral and canine prostate convert ^{14}C-3α-diol predominantly to 5α-dihydrotestosterone, and minimally to hydroxylated products. When 8.5 nM ^{3}H-3β-diol was exposed for 21 hours to canine prostate in serum-free organ culture, 65% of the radioactivity in both explants and medium was found in 7- and 6- hydroxylated derivatives. Under these conditions 4–5 times as much specifically bound ^{3}H-5α-dihydrotestosterone (to high-affinity cytoplasmic and nuclear receptors) was generated from either testosterone, 5α-dihydrotestosterone or 3α-diol ^{3}H-substrates than from ^{3}H-3β-diol. Thus, in considering the 3β-diol effect at the target site, one must take into account the compartmentalization of the diol *per se* and the derived 5α-dihydrotestosterone, and its rapid transformation to hydroxylated terminal metabolites. Furthermore, Dr. Moore may have tested the rat ventral prostate cytosol for 3β-hydroxysteroid oxido-reductase activity, but Dr. Robel found it in organ culture of rat ventral prostate. We found it in rat ventral and canine prostate, human prostate adenoma and in canine epididymis and perianal glands, but in our hands it is an unstable enzyme and lost activity relative to 3α-hydroxysteroid oxido-reductase on homogenization and cytosol preparation. 3β-diol is poorly converted to 5α-dihydrotestosterone by minced prostate tissue without co-factor supplementation. I brought the matter up here because of the aforementioned new work, using canine prostate explants and high SA ^{3}H-3β-diol, which requires only partial modification of my previous statement.

WILSON: 3β-hydroxysteroid oxidoreductase is present in red cells in almost all species. Unless contaminating red cells are excluded, activity can be measured in every tissue, but it is almost impossible to show 3β-hydroxysteroid dehydrogenase in the rat prostate when it is free of red blood cells.

COFFEY: Let me ask Dr. Bruchovsky. Have you studied labeled 3β-androstanediol to see whether nuclear up-take occurs and whether it is converted to DHT or not?

N. BRUCHOVSKY: Two years ago we discussed this point in San Antonio. And at that time Dr. Ofner insisted that 3β-diol could not be formed in prostate. At that time I did mention that 3β-diol, when injected into rats, resulted in the formation of dihydrotestosterone in the prostate and in nuclear localization. That doesn't necessarily mean that the prostate itself forms the dihydrotestosterone from 3β-androstanediol. Looking at the problem in another way, when we examined prostate after injecting dihydrotestosterone, about 10–20% of the total androstanediol is 3β rather than 3α. That's quite clear from a variety of tests, including recrystallization, high speed liquid chromatography, gas chromatography and also TLC. The interesting thing is that in cell free systems with optional concentrations of TPNH, only 3α-androstanediol is formed from radioactive dihydrotestosterone. So, in a cell free system, 3β-androstanediol cannot be detected. If you go in the reverse direction, using 3β-androstanediol as the substrate and drive your system with DPN, not TPN, dihydrotestosterone formation takes place. The point that red cells trapped in prostate tissue preparations contribute to androgen metabolism must certainly be considered.

P. ROBEL: With ^{3}H-dihydrotestosterone as substrate, we have shown a 3β-hydroxysteroid dehydrogenase activity in rat ventral prostate cytosol. This activity was very small compared to the 3α-hydroxysteroid dehydrogenase (STEROIDS *23*, 291–299, 1974). These cytosol preparations might contain some blood proteins, but in addition we have done many organ culture experiments with small washed explants where blood or plasma contamination should be minimal, including several experiments with completely defined culture medium with serum. However there is a discrepancy between the whole tissue experiments where a significant amount of 3β-androstanediol is found and the cytosol experiments where we found a very small formation of 3β-androstanediol. Conversely, is 3β-androstanediol converted into dihydrotestosterone? We have investigated this very carefully in the steady state with the use of organ culture superfusion. When a 10 nM concentration of 3β-androstanediol is superfused, the amount of dihydrotestosterone found in the tissue in only 3% of that found when 10 nM dihydrotestosterone is superfused. Since dihydrotestosterone is still active at very low (0.1 nM) concentration, this small conversion of 3β-androstanediol into dihydrotestosterone might explain, at least in part, the activity of the former compound. In addition, we have observed a very strong specific binding of 3β-androstanediol to the rat ventral prostate microsomes (Biochim. Biophys. Acta, *373*, 1–9, 1974). This leaves open the possibility that the 3β-androstanediol may be active itself, possibly at the translational level, and this possibility deserves further investigation.

R. NERI: We have shown that *Sch 13521* (flutamide) is as active as cyproterone acetate in

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blocking androgen action based upon a number of biological assays. I am gratified that Dr. Coffey corroborated this in his rat model measuring the rate of DNA synthesis. Dr. Peets and I have shown that the compound in vivo blocks the androgen receptor site in the rat ventral prostate. However, if flutamide is given in vitro, the ability to block binding of androgen to the receptor is reduced. We suspected that flutamide's antiandrogenic activity may be attributable to an active metabolite. This view was shared by Drs. Liao, Mainwaring, Fang and Sandberg as a result of their studies with flutamide. Katchen in our laboratory reported on the disposition of flutamide in men follow-a single oral dose (J. Clin. Endo. and Metabl. *41*: 373–379, 1975). The rapid conversion of flutamide to the hydroxylated form, *Sch 16423*, and the high plasma levels of *Sch 16423* suggest that this might be the active form of flutamide. Biological studies indicated that *Sch 16423* has 1.5 times the potency of flutamide. *Sch 16423* inhibited the uptake of tritiated testosterone and dihydrotestosterone by rat ventral prostate in both in vivo and in vitro.

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PROSTATIC CELLULAR ALTERATIONS WITH AGING

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As a general rule, when cells grow old or when they reside in an aging animal, they change their shape and their rate, if not their quality, of activity in parallel with their host. Franks found this true of the prostate when he compared the glands of 5-month and 2-year old mice (1). In contrast to the uniform, well-structured epithelium in the glands of the young adults, the cells of the aged animal were shrivelled so that they lost height and were crowded to the point that they bulged into occasional papillae within the acinae. Within these areas the cells appeared hyperplastic with some mitoses; the nuclei were variable in size. Vacuolation in the Golgi area was irregular, and diffuse and lipoid granules, more extensively studied by Harkin (2) and Brandes (3), were frequently present in the supranuclear regions. This expanded volume of Golgi vacuoles was reminiscent of the dilation of ergasto-plasmic sacs seen by Harkin one day after castration (4).

Prostates from animals of all ages seemed to thrive when the glands were cultured for 21 days in the rich medium provided by Franks (1). Only the crowding of cells and the consequent projection of the acinar lining into the lumen distinguished glands of 2-year olds from those of young adults. The presence of testosterone propionate in the medium had little discernible effect on the pattern of growth of donor cells of the two ages.

In contrast to mice, there is little evidence of senescence of the cells in the prostates of most elderly men at the light microscope level (5). A succulent, hyperplastic gland is so common in this age group as to seem to be the normal product of ontogeny, in spite of the disease that it imposes.

The nodules of benign hyperplasia (BPH), as described by Mao et al (6), consist of acini embedded in a fibromuscular stroma. Epithelial cells, ranging from low cuboidal to tall columnar, arise from a basement membrane to line the acini and contribute secretory products to its lumen. There are basal (or myoepithelial) cells insinuated between the more active epithelium. Secretory activity, both apocrine and merocrine, is qualitatively identical to that of normal prostate but is quantitatively less. Scanning electron microscope studies by Clark et al (8) may provide an explanation why there is less secretion by the hypertrophic gland: Between 80% and 90% of the cells of a control specimen are low columnar to cuboidal epithelium and are covered with microvilli. The remaining 10% to 20%, which are tall, secretory columnar epithelium, have a distinct, crater-like stroma, lined with microvilli. By transmission electron microscopy, the craters are seen to be the openings of the intracellular canaliculi from which secretion emerges. In the BPH specimens, besides the aforementioned cell types, are a group of cells that are devoid of microvilli, and probably do not secrete. They are reminiscent of BPH cells described by Mao et al (6) and perhaps the involutional epithelium of McNeal (9).

Besides these apical changes associated with aging, Brandes et al (10), Fisher and Jeffrey (5), and Kirchheim and Bacon (11) described striking dilata-

This work was performed under the support of VA Project #2746-01

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tion of the clear spaces between the basal regions of hypertrophic acinar cells. There were numerous evaginations suggestive of pinocytotic activity. Such configurations reminded the authors of similar subcellular responses observed in mouse uterine epithelium at the peak of estrogenic stimulation. The possibility was considered that in BPH there may be an increased sensitivity to estrogens due to the embryological relationship of the periurethral region of the prostate to the Müllerian ducts. The possibility that BPH is an estrogenized state, which harkens back to the suggestions of Lacassagne (12) that there is a shift in hormonal balance with aging are compatible with the increase in the estradiol/testosterone ratio found by Rubens et al in males over 65, relative to those under 50 (13). By most criteria, however, the aged prostate is well supported by nutrients and hormones. Its morphology best compares with the androgenized ventral prostate shown by Woodruff and Perez-Mesa (14) and not to that of the gland of the estrogenized animal. Siiteri and Wilson (15) reported that the aged prostate, and especially the nodular hyperplastic region, is rich in dihydrotestosterone (DHT) and the 5α-reductase required to make more DHT from available testosterone (T). If the tissue were deranged by estrogens, most of us agree (Shimazaki et al, 16; Farnsworth, 17) the 5α-reductase would be inhibited. Both on biochemical (18,19) and histochemical grounds (20,21,22) there is no depletion of acid phosphatase. This is presumptive evidence both of sustained hormonal support and of the capability to generate and deploy the energy needed for synthesis of this specific protein.

Thus, the aging prostate in man does grow in size, does change in structure and secretory activity but *does not* seem at all deprived of hormonal support in spite of a dwindling absolute and relative (to estrogen) level of circulating androgen. We need to know if these conflicting circumstances are the result of an intrinsic change in the sensitivity of the cell due to compensatory shifts in its environment. Also, if BPH is a reflection of intrinsic transformation of the cell, is the change the product of normal ontogeny or an extracellular agent?

Lasnitzki's (23) studies of mouse prostate in organ culture argue for intrinsic changes with aging, just as do those of Franks(1). Ventral prostates from 4–6 weeks old animals exhibit a greater requirement for androgen support than do those from 3–6 month old donors. A level of testosterone that maintains young glands induces intense hyperplasia in the older group. Sensitivity to estrogen also shifts dramatically: Estrone, at 2 to 4 ug/ml, induces hyperplasia and metaplasia of the young cell but collapse and atrophy of alveoli accompanied by stromal hypertrophy in cultures from the older animals. Rohl (24) found that cultures of human hypertrophic prostate were insensitive to levels of androsterone that stimulated vigorous proliferation of differentiated cancer cultures. Brehmer et al (25) and McMahon et al (26,27) obtained similar results. Schroeder and Mackenson (28) found that monolayer cultures of both BPH and cancer cells grew equally well in androgen-free and testosterone-containing media but that without testosterone there was no production of acid phosphatase. Riemann et al (personal communication) have approached the study of aging by observing ultrastructural changes in monolayer cultures derived from benign hypertrophic prostate cells for up to 10 transfers. In the absence of hormonal supplement, they observe progressive vacuolation and the proliferation of lysosomes leading to intracellular digestion. Perhaps this study tells more about castration changes than about changes in an aging animal. Helminen et al (29) have described the emergence of increasing cathepsin levels in parallel with declining acid phosphatase activity in the cells of rat prostates undergoing castration-induced involution.

Mainwaring's (30) in vivo results are in keeping with those of Lasnitzki: He found that the enzymes and fundamental cellular constituents of the prostates of 9 month old mice were unaffected by subcutaneous implants of testosterone that were stimulatory to growth in glands of older animals.

These results raise the question as to whether the cells become more sensitive to androgens with age and whether these changes are due to modifications in the availability or metabolism of the hormones in the blood?

Moore and Wilson (31) found recently that 5α-reductase is induced by androgens. This could account for the increased "sensitivity" of the mature as compared to the immature gland since the older tissue would have been subjected to a rising level of inducer steroid not yet available to the immature gland. By the same reasoning, the decrease in plasma testosterone concentration in the aging male should undo or deplete the enzyme complement and thus lower the effectiveness of the remaining androgen available. In fact, Nozu and Tamaoki (32) (Table 1) found this to be true. Upon separation of smooth from rough microsomes of rat ventral prostates they learned that both the specific activity and content of 5α-reductase in the rough fraction from 3-months old animals were greater

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TABLE I—Intermicrosomal Distribution of the 5α-Reductase Activity of Rat Prostate

	Preparation	Rough-MS/smooth-Ms	RNA/protein	5α-reductase activity
Expt. 1	Total-Ms		0.09	157
(3 months old)	Rough-Ms		0.12	176
	Smooth-Ms	4.03	0.01	129
Expt. 2	Total-Ms		0.15	123
(3 months old)	Rough-Ms		0.16	122
	Smooth-Ms	3.75	0.03	87
Expt. 3	Total-Ms		0.05	36
(12 months old)	Rough-Ms		0.13	51
	Smooth-Ms	0.78	0.02	48

than in the smooth fraction. In preparations from 12-months old rats the amount of enzyme in the rough fraction was only about 80% of that in the smooth, and the specific activity of both fractions was about equal and only 25% of that found in the microsomal protein of the young animals' glands. Obviously, biosynthesis of reductase is far less in the older animal's prostate.

Another determinant of affinity and responsiveness of the prostate cell is the complement of steroid-specific receptors. Shain and Axelrod (33) find that the 10–11S dihydrotestosterone-specific binding protein of the young rat's prostatic cytosol disappears completely in most animals of each of two strains when these animals reach 12 to 24 months of age. These investigators took pains to show that this loss is not the result of a dissociation of the protein to a non-specific 3.5-4S moiety. Finally, Armstrong and Bashirelahi (34) have found that a 3.5S protein that specifically binds estradiol appears in the ventral prostate of retired breeders.

Siiteri and Wilson (15) showed that the concentration of dihydrotestosterone was increased in the benign hyperplastic human prostate and that especially high levels were contained in the 5α-reductase-rich nodules. Farnsworth (35) was able to complement these findings with the demonstration that levels of testosterone, androstenedione and dehydroepiandrosterone in nodular hyperplastic tissue ranged from 5 to 7-fold their respective concentrations in tissue-matched plasma, a positive indication of the tissue's ability to take up the hormone. Becker et al (36) and Briggs (37) have confirmed these impressions by showing incorporation of infused radiolabelled T and DHT into subsequently excised hypertrophic glands. Now, Giorgi's group (38), using their superfusion techniques, can show that human hypertrophic tissue has such an excessive number of "non-specific" binding sites that it binds more testosterone and dihydrotestosterone than do either normal human or canine prostates. Also, as expected, a selective binding protein has been isolated from the cytosol and the nuclei of BPH tissue by Hansson et al (39).

We can conclude from these findings that the aged, hyperplastic prostate is at the top of its form and not at all comparable to the atrophic gland of the aging rat, except that in both the level and character of intracellular steroid are determined by receptor proteins. As to the mechanism through which steroid aquisitiveness is enhanced, there are at least three possibilities: It may be that the prostate cell is programmed to generate more receptor as it ages and that in man this is an open-ended mechanism with no provision for a stage of senile atrophy such as we have seen in the rodent.

A second possibility is that, like the 5α-reductase, the receptors are induced by rises in the circulating hormone level. At first thought, this mechanism seems unlikely for, as already mentioned, the hyperplasia, about which we are concerned, arises at a time of falling androgen concentration, when the estradiol/testosterone ratio is increased to twice the level encountered in the man under 45 years of age. It is premature, however, to discard the possibility that an equal or even greater effective concentration of circulating hormone may be acting on the prostate cell. The potentially effective concentration of testosterone is that part of this steroid pool that is not tightly bound to the sex hormone binding and cortisol binding globulins of the plasma but travels in loose association with albumin. We (Farnsworth and Brown, in preparation) find that this apparent free testosterone concentration in the plasma of 13 BPH subjects (9.8 ± 1.1 ng/100 ml) is neither more nor less than in a group of 13 healthy young normals (11.6 ± 0.7 ng/100 ml). For this fraction to be actually effective, it must gain access to the target cell. In 12 benign hypertrophic glands in which we have measured by competitive protein binding the testosterone, dihydrotestosterone and androstandiol,

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the aggregate mean concentration is 1500 ng/100 gm, representing about a 130-fold concentration of the apparent free testosterone concentration in matched plasmas. As far as plasma levels are concerned, Mahoudeau et al (40) have seen no difference between plasma levels of testosterone, dihydrotestosterone or the ratio of the one to the other in BPH subjects as compared to age-matched controls. Vermeulen's group (41) observed that, due to rises in the sex hormone binding capacity in men over 50 years of age, the apparent free testosterone concentration does fall significantly.

The additional influence of circulating peptide hormones, especially prolactin, to modulate steroid uptake through their effect on the cell surface must also be recognized. Shiu and Friesen (42) showed that prolactin-specific receptors line the cells of the mammary gland. Now, the radio-autographic work of Rajaniemi et al (43) reveals a qualitatively similar affinity of ventral prostate epithelium for ^{125}I-labelled prolactin. At appropriate concentrations, prolactin synergizes with both testicular (44) and adrenal (45) androgens in the support of prostatic growth, acting, it appears (46), by increasing the affinity of the cells for these steroids (47). Most of the two principal adrenal androgens, DHEA and androstanediol, is carried in the blood as inaccessible, water soluble sulfate, but this conjugate can be cleaved by a potent sulfatase in the prostate whose activity varies inversely as the concentration of testicular androgen (48). Thus, the depletion of free testosterone in the aging male should permit increasing levels of free DHEA and androstenediol to become available to the prostate. Finally, the rise in estrogen which raises sex hormone binding capacity and inhibits 5α-reductase, also increases prolactin secretion. Harbitz (49) and Haugen (50) find that in patients over 65 with BPH, there is, accompanying a rapid rate of testicular atrophy and increase in the numbers of gonatrophin-producing pituitary basophils, an even more striking fall in hypophyseal acidophiles, the cells that secrete prolactin. Does this indicate an increasing rate of delivery of prolactin to the blood stream?

The third possible way to modify the prostate cell is through a traumatic agent, e.g., perhaps BPH is a precancerous state. (Table 2) Armenian et al (51) found that the age-adjusted death rate from prostate cancer was 3.7 times higher in a group of 296 BPH cases than in 299 age-matched controls. (Table 3). Retrospectively, patients previously hospitalized for a noncancerous prostatic condition had over 5 times more risk of readmission with prostatic cancer than matched controls.

In support of the argument that both BPH and cancerous prostate cells may have undergone a common transformation is Ablin's finding (52) that they are both depleted of normal prostatic tissue-specific antigens and the report of Avis et al (53) of antigenic cross reactivity between prostatic hyperplasia and adenocarcinoma.

It is hard to determine if cellular immunity is also affected since authors tend to use the lymphocytes of BPH patients as controls. Nevertheless, McLaughlin and Brooks (54) and Catalona et al (55,

TABLE II—Age-Adjusted* Death-Rates Per 1000 Person-Years and Proportional Mortality From Prostate Cancer and all Cancers of the Study Groups and New York State, Exclusive of New York City

Cause of death	B.P.H. cases	Controls	N.Y. State 1960
Age-adjusted mortality-rates			
All causes	105·5	113·5	87·3
Prostate cancer	10·1	2·7	2·0
All cancers	26·2	20·4	12·4
Cancers other than prostate	17·9	19·3	Not available
Prostate cancer as % of all causes	9·57	2·38	2·30
Ratio of death-rates, study groups to N.Y. State (1960)			
All causes	1·21	1·30	--
Prostate cancer	5·05	1·35	--
All cancers	2·11	1·65	--

*Using the total study population as the standard.

TABLE III—Frequency Distribution and Results of Matched-Pair Analysis of Antecedent Hospital Admissions for Non-Cancerous Prostatic Conditions

Interval (yr.)*	No. with hospital admission		Matched-pair analysis		
	Prostate cancer (N=290)	Controls (N=290)	Relative risk	x^2	P
0	10	3	3·3	3·8	N.S.
1	22	5	3·3	10·7	<0·001
2–3	21	2	10·5	15·7	<0·001
4–5	11	4	3·3	3·8	N.S.
6–10	15	4	4·7	7·1	<0·01
11+	8	3	2·7	2·3	N.S.
6 or more only	23	7	3·7	9·1	<0·001
All	87	21	5·1	44·5	<0·001

*Between index hospital admission and antecedent admission for non-cancerous prostatic condition.

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56) do find that lymphocyte concentration and reactivity tend to diminish in even localized prostatic carcinoma.

Gonder (57) has proposed an alternative mechanism for transforming the aged prostate cell to the hyperplastic state. He suggests that the cells may undergo autoimmunization induced by antigens liberated by prostatitis. This is an attractive hypothesis from two points of view. It suggests benign hyperplasia need not be an early manifestation of cancer even if it may predispose the cells to carcinogenesis. Secondly, it facilitates accounting for the high incidence of BPH, in that some form of chronic prostatitis may afflict as large a proportion of the male population.

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DISCUSSION

J. GELLER: I was interested in your comments that there is an extracellular as well as an intracellular hormonal environment. And, it is the latter, the intracellular environment, that I think may be most productive in leading us to an understanding about BPH. We have developed new techniques for the measurement of intracellular androgen metabolites, and I would like to report that we obtained values for BPH very similar to that reported by

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Sitteri and Wilson, namely, in the range of 5–6 nanograms of dihydrotestosterone per gram. We are also able to measure androstanediol which is new. We didn't separate 3α from 3β, but we do find about 9–10 nanograms per gram of the diols in the normals, whereas relatively low levels of 2–3 nanograms per gram were present in hypertrophic prostate which makes us think that there is a block in the conversion of dihydrotestosterone to androstanediol.

W. E. FARNSWORTH: We have not looked at normal prostate but have only compared BPH with carcinoma. We find that there is a significant difference between the two types of tissue. The level of apparent free testosterone in plasma seems to be lower in carcinoma patients, but the sum of testosterone, dihydrotestosterone and diols (We didn't separate 5α-androstane-3β-diol from 5α-androstane-3α-diol.) in the cancer tissue is significantly higher than in BPH samples at the 5% level. This higher ratio of tissue to plasma androgens in cancer tissue suggests a greater capability to concentrate steroid from the blood.

S. G. KORENMAN: We heard this morning that a large fraction of BPH is stromal hyperplasia rather than an increase in the number of epithelial cells, and Dr. Geller just said that there is a tremendous increase in the concentration of certain steroids and a decrease in other steroids. In these various studies what assumption is made about the cell type, or whether it is lumped together in the assumption that it is a uniform tissue, which it certainly is not.

FARNSWORTH: We recognize the importance of this problem and have attempted to cope with it in two different ways. The first is the use of the Franks' Press, with which one is able to obtain from the human prostate a relatively pure preparation of epithelium with about a 10% yield. The problem is that it takes a lot of prostate to get enough epithelium for analytical work. The second approach is based on the finding by Nilsson, Schueller and Staubitz (Invest. Urol. *11*: 145–148, 1973) that β-glucuronidase activity in benign hypertrophic prostate is confined to the epithelial cells. What we have done is to work up what we call an "epithelial index": Tissue samples are assayed for β-glucuronidase activity per unit of protein. It is then assumed that glands with relatively high glucuronidase specific activity contain more acinar and less stromal tissue than those of low specific activity; e.g., a sample exhibiting 50 units per gram is twice as rich in epithelium as one with only 25 units per gram. This principal has been applied to the analysis of prostaglandin $F_{2\alpha}$ in prostate samples (Farnsworth and Wilks, Prostaglandins *9*: 67–74, 1975). We found that the prostaglandin concentration varied inversely as the glucuronidase specific activity, suggesting that the prostaglandin must accumulate in the stroma. It is of interest that this seems also to be true by immunofluoresence. Further effort to determine the correlation of the ratio of epithelial hyperplasia to stromal hyperplasia to glucuronidase specific activity is in progress.

M. TANNENBAUM: Well, just as a comment, why examine autopsy material? There are many published autopsy studies on BPH over the years. One initially sees in autopsy material a so-called nodular stromal hyperplasia that looks almost like fetal mesenchyme. This can begin in the late 20's and early 30's. However, if surgical material is examined, so-called nodular hyperplasia that is purely stromal can be separated easily from that which is epithelium. The nodular stromal hyperplasia is periurethral. In a 30–40 gram prostate, 2–4 grams of pure stromal tissue can be dissected with very little epithelium. If one wants to elucidate the etiology of BPH, pathologically it begins as a mesenchymal lesion that later has epithelium grow into it.

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BENIGN PROSTATIC HYPERPLASIA: GROSS AND MICROSCOPIC ANATOMY

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Giambattista Morgagni of Padua (born 1682, died 1771) was one of the founders of modern pathology, and he was not only a pathologist but also a very keen clinical observer. He clearly recognised the fat, plethoric type of patient likely to suffer from prostatic disease and described the common site of origin of enlargement of the gland as can be seen in the following extract "If you attentively examine those examples which I have pointed out from the Sepulchretum, and that which I have produced above, from Valsalva and Mine; you will observe that they were all from old men; and in a like manner, if you examine all my observations in which there was the beginning of a caruncle you will find that this was found to grow out in the very middle of the internal and upper circumference of the gland, posteriorly; but whether all these things happened by chance, or otherwise, future observations will show" (1). You see from this comment that the basic point concerning the pathology of benign hyperplasia was already well established.

There is little doubt that the specific features of the pathology of the prostate depend on the anatomical structure and embryological development of the gland. Although there is some disagreement about the precise terminology of the various prostatic gland areas (2) there is no dispute about the basic facts.

The normal adult gland

There are four groups of tissue contained within the prostatic capsule—the urethra and small evaginations from it, the genital tract including ejaculatory ducts, prostatic utricle and at times the lower ends of the seminal vesicles, the peri-urethral glands, and the prostate proper. The gland surrounds the urethra which, in cross-section, is triangular with its apex pointing forwards. The posterior wall projects into the lumen forming a central ridge—the verumontanum; the larger prostatic ducts open into the grooves on either side. The prostatic utricle opens onto the summit of the ridge with the mouths of the ejaculatory ducts on each side.

Anatomically, the prostate is generally divided into five lobes—posterior, middle, anterior and two lateral—related to the structures which pass through it. The posterior lies behind the urethra and deferential canal. The middle lobe lies between the urethra and deferential canal, and the anterior lobes is in front of (i.e. anterior to) the urethra. The lateral lobes lie on either side of the urethra. These anatomical lobes were said to correspond to the groups of glands which were described by Lowsley (3) in the embryo, but no division into separate gland groups of this type is possible from the last months of gestation and in post-natal life (4).

The distribution of the normal prostatic glands

On cross section there is no definite lobar pattern, but three concentric regions of increasing size from within out can be distinguished (Figs. 1 & 2).

The main mass is composed of long branched "external" or outer glands, leading into ducts which curve backward to enter the postero-lateral sulci. This thick outer layer invests a smaller mass of

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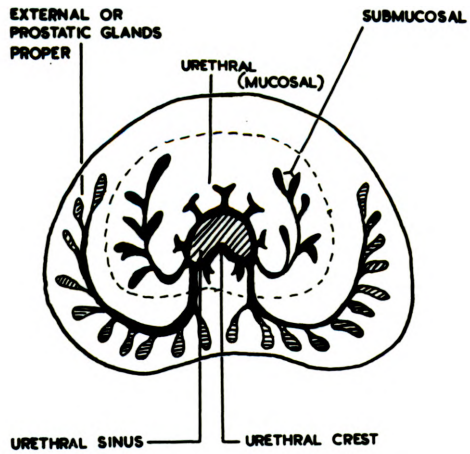

FIGURE 1. The distribution of the normal prostatic glands slightly modified (from Grant, J. C. B., A Method of Anatomy, 1952). The urethral (mucosal) and submucosal glands together form the inner group which is separated from the outer gland group by an inconstant capsule.

FIGURE 2. A transverse section of prostate showing the main mass of outer glands, submucosal glands, and urethral (mucosal) glands. There is no sharp line of separation between submucosal and outer glands in this section. Age unknown. (X 1 approx)

inner secreting elements and is separated from it by an inconstant capsule. The inner group consists of a larger set of fairly long branched submucosal units, the ducts of which open into the postero-lateral grooves, and a smaller mucosal group of short glands surrounding and opening directly into the urethra. The ducts of some sub-mucosal glands may open into the main duct of an external gland, near the urethra. The acini of adjacent submucosal and external prostatic glands are often interdigitated and on cross section a clear line of separation may not be apparent. The inner glands are sometimes few in number in youth. The urethral glands are well marked but the submucosal glands appear as a narrow band of small acini roughly parallel with and in front of the urethra. The inner glands usually become more prominent with age, and the separation of inner and outer gland groups becomes more marked (Fig. 3). Occasionally, the inner glands may be absent (5 and 6).

The Epithelium

The epithelium is tall columnar, sometimes with an underlying basal layer of flattened cells, and there is a definite secretory cycle. Histologically, the epithelium of both inner and outer gland groups is similar. The duct epithelium is also columnar except near the outlet where it becomes transitional.

The stroma

The stroma is made up of collagen and elastic tissue with many smooth muscle fibers, arranged around the ducts and acini (peri-ductal and peri-acinar) and between the gland lobules (inter-lobular). Here it forms fairly well defined septa which radiate from the colliculus where the muscle forms a thick ring around the urethra—the internal

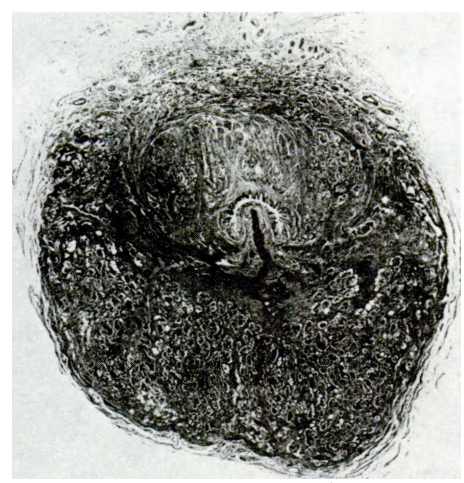

FIGURE 3. Transverse section of prostate showing indefinite capsule between inner and outer gland groups. Age 51 years. (X 1 approx)

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sphincter of the bladder. Around the acini there is a thin zone of collagen with a few elastic fibres and a band of smooth muscle so arranged that contraction would appear to reduce the size of the lumen equally from all sides. There is a thick network of elastic fibres around the larger ducts and urethra. At the periphery the stroma is condensed to form a capsule of circular (circumferential) strands of collagen, elastic tissue and smooth muscle. A few voluntary muscle fibres are often found particularly anteriorly and laterally.

Benign nodular hyperplasia: the site of origin of the nodules

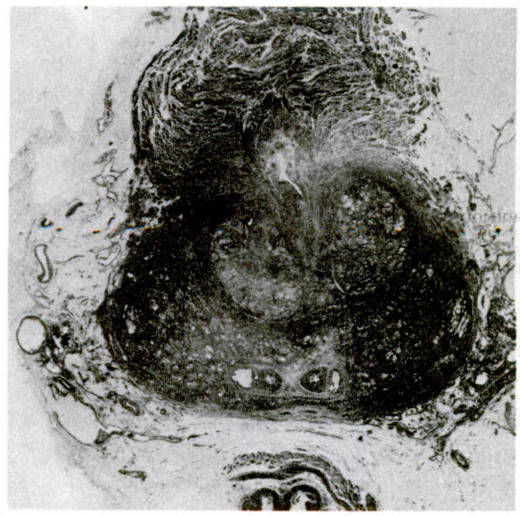
Benign nodular hyperplasia of the prostate is due to hyperplasia of the inner group of glands but its exact site of origin was not clearly defined until the end of the 19th century. In 1894, Jores (5) described groups of submucosal glands on the trigone, vesical neck and in the urethral walls just below the bladder neck. These glands, though few in early life, increase in number with age. Occasionally, they may be absent. He also showed that some submucosal and median enlargements of the prostate arose from these glands.

In 1925, however, Reischauer (7) claimed that the initial change occurred not in the glands but the periglandular stroma near the urethra. He believed that fibromuscular nodules developed in the stroma and that these nodules stimulated epithelial proliferation in the neighbouring glands which then penetrated the nodules from the periphery. He showed that many of these fibromyomata contained no epithelium, and he correlated the statistics of earlier observers (8) on the frequency of glandular, fibrous and mixed types of prostatic hyperplasia with the inconstancy of distribution of the submucosal glands in the normal prostate. Only when the nodule arose in a gland free area did it remain purely stromal. This theory has been supported in a number of careful papers (9, 10 & 11).

My own findings (12) show that both stromal and epithelial hyperplasia may occur, alone or together, and it is impossible to say that the one occurs before the other. All the tissues of the inner group of glands appear to respond by hyperplasia to the stimulus which causes benign nodular prostatic enlargement.

When the nodules are small (Figs. 4 & 5) the outer prostate makes up the greater part of the gland, but as the nodules increase in size the tissues of the outer prostate become compressed, and form a thin pseudocapsule partially surrounding the enlarged hyperplastic inner glands (Fig. 6). To the naked eye, the creamy white benign nodules are clearly separated from the outer glands (Fig. 7). If the so-called middle lobe is involved it may project into the bladder and cause a ball-valve type of obstruction (Fig. 8). In the usual operations of prostatectomy the nodules are shelled out leaving the compressed outer prostate—the so-called "surgical capsule"—behind.

The hyperplasia is characteristically nodular, and both epithelium and stroma (muscular and fibrous but not elastic) take part in the process in similar



FIGURES 4. and 5. Transverse sections of prostate showing benign nodular hyperplasia arising in the inner part. (X 1 approx)

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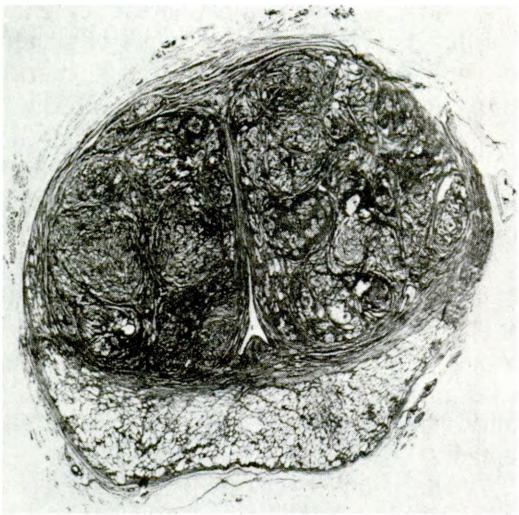

FIGURE 6. Transverse section of prostate showing large hyperplastic nodules in the inner part of the gland. The tissues of the outer prostate are compressed around the edges. The large nodules are made up of numbers of smaller ones. (X 1 approx)

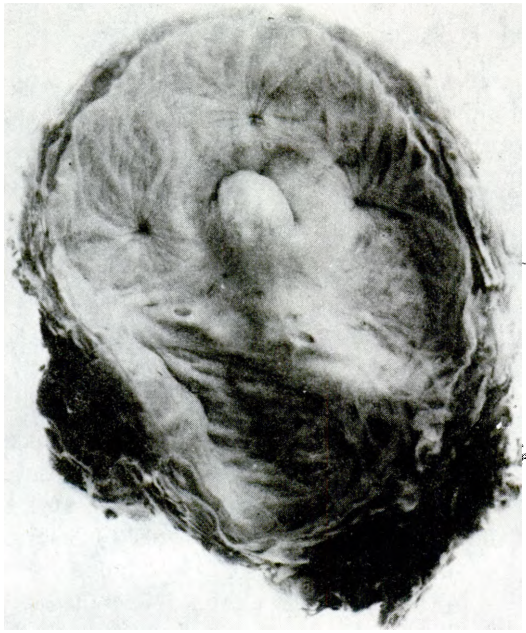

FIGURE 8. "Ball-valve" projection from middle lobe, obstructing urethral orifice. (X 1 approx)

fashion to changes in the breast except that the elastic hyperplasia, so often seen in that organ, is not seen in the prostate.

Epithelium of either duct or acinar type and muscular and fibrous stroma in any combination take part in the formation of the nodules. The hyperplastic collagenous stroma is, however, always the loose pale-staining stroma propria (i.e. the subepithelial periductal and periurethral stroma). It is impossible to say which element appears first in the nodules, and indeed it is obvious that the change may begin in either epithelium or stroma. Although apparently well defined the nodules almost always merge with the surrounding gland tissue at some point on their circumference.

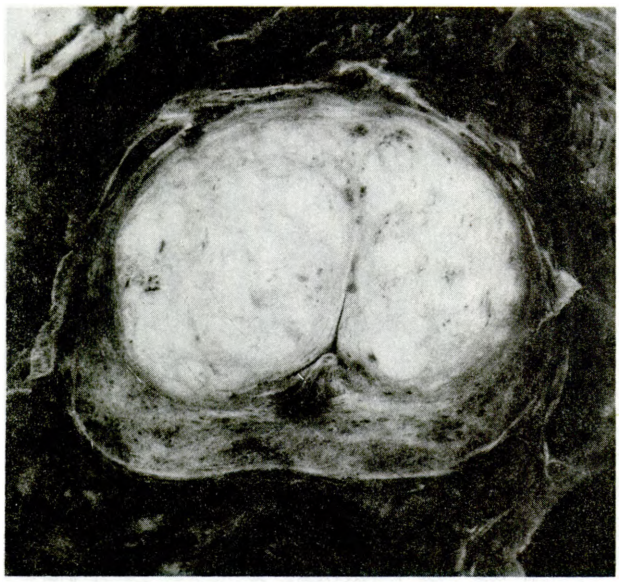

FIGURE 7. A specimen showing similar changes to those seen in Figure 6. The outer prostate is darker in colour that the hyperplastic nodules, which can be seen to be made up of many smaller ones. (X 1 1/4 approx)

Morphologically there are five types of nodule:

1. the stromal (fibrous or fibrovascular) nodule,
2. the fibromuscular nodule,
3. the muscular nodule ("leiomyoma"),
4. the fibroadenomatous nodule,
5. the fibromyoadenomatous (fibromyoepithelial nodule).

The stromal (fibrous and fibrovascular) nodule

The fibrous nodule must be distinguished from fibrous scars of infarcts or healed prostatitis. These latter may occur anywhere in the prostate, have an indefinite margin and often contain scattered strands of smooth muscle, elastic tissue and metaplastic epithelium (the remains of ducts and acini), lying in dense fibrous tissue with relatively few

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fibrocytes. The collagenous stroma stains specifically with the usual collagen stains.

The true stromal nodules are found only in the subepithelial tissues around the urethra. These stromal nodules occur above the distal end of the verumontanum and are clearly demarcated to the naked eye from the surrounding tissues, standing out as round or ovoid white areas in the pinkish grey periurethral tissues. While as a rule small, i.e. up to 5mm. in diameter, there is a considerable variation in size, some being microscopic while others may reach 2cm. in diameter, though rarely. Histologically, these stromal nodules (Fig. 9) are not as clearly defined as the gross appearances suggest and almost always merge with the surrounding tissues at some point on their circumference. The nodes are made up of a meshwork of fine fibrils with groups of elongated spindle cells or of flat stellate cells (Fig. 10) with many short interdigitating branching processes arranged around small vascular spaces. The nuclei of these cells are large ovoid and vesicular, and the endothelial cells lining the blood channels are plump and project into the lumen. These whorled cell groups are often arranged in closely packed layers around a well formed blood vessel. The fibres are stained by the silver methods for reticulin, and only the coarser fibres stain selectively for collagen. Elastic tissue is generally absent from the nodule but may be found about its margin (Fig. 10). Retrograde injection of the prostatic veins with a barium suspension of standard particle size (0.2 u) fills all the prostatic veins up to the very small post-capillary venules, but the vessels of these nodules do not fill. They are therefore presumably derived from the arterioles or arterial capillaries. These stromal nodules are morphologically similar to the loose peri-utricular tissues, but there is a dense elastic network around the utricle. Both resemble primitive mesenchyme.

The fibromuscular nodule

Again many fibromuscular nodules represent the end result of an infarct with partial replacement of muscle and loss of prostatic epithelium. Others are infiltrated with inflammatory cells particularly histiocytes and plasma cells and are perhaps best interpreted as a nodular granulomatous prostatitis.

The larger nodules are similar to the muscular nodules but with a greater connective tissue content, and the distinction between these two is purely arbitrary. The smaller nodules resemble the

FIGURE 9. Two small stromal nodules near the urethra. The lower nodule has an ill defined margin and merges with the surrounding tissues above and below. Well formed blood vessels can be seen in the nodules. (X 40)

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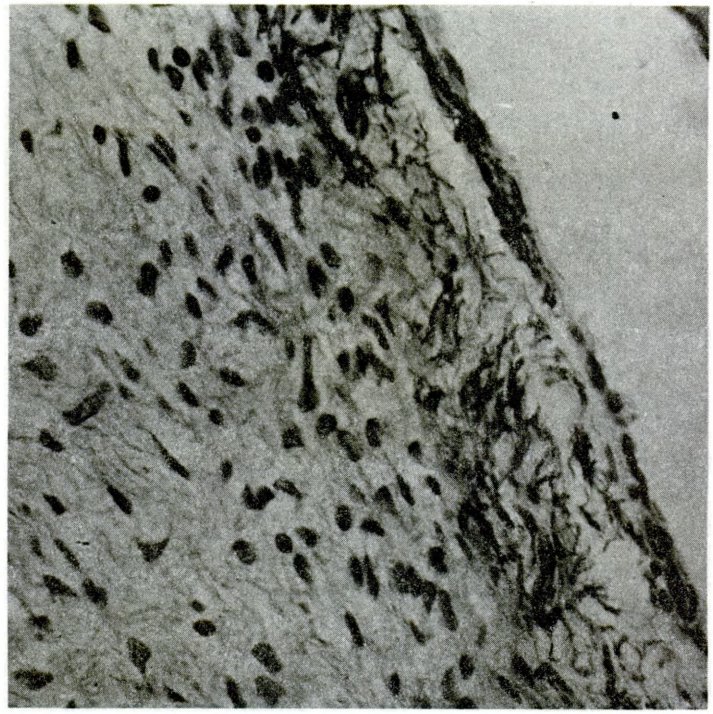

FIGURE 10. The edge of a nodule showing elastic fibrils (black) about its margin (Orcein and haematoxylin) (X 540)

small stromal nodules but contain strands of smooth muscle (Fig. 11), sometimes well differentiated but often not, the cells having some of the morphological characters and staining reactions of both the stromal cells and plain muscle. Thus, it would seem that these stromal cells may be able to differentiate into smooth muscle cells. They may be derived from vascular pericytes which have this developmental potential. The mixed cellular pattern has been confirmed by electron microscopy (vide infra). This type of fibromuscular nodule cannot be distinguished from the true stromal nodule with the naked eye.

The muscular nodule

The pure muscular nodule is uncommon. It is possible but unlikely that these are tumours, i.e. neoplastic. More probably most represent a localised muscular hyperplasia with few or no included epithelial elements. They are as a rule large and often single (i.e. unilateral) forming the whole of one side of a symetrically enlarged nodular hyperplastic prostate. To the naked eye the cut surface is pale grey and glistening, with interlacing bands running across it. The muscular nodule tends to bulge irregularly above the surface particularly at its centre.

Histologically it is made up of whorled bands of typical smooth muscle fibres with relatively scanty collagenous stroma. This whorled appearance is almost always absent in the small fibromuscular nodules. Elastic tissue is very scanty or absent, yet abundant in the surrounding prostatic stroma.

The fibroadenomatous nodule

Macroscopically these nodules have a coarsely granular cut surface with small pale-yellowish epithelial areas scattered over the grey background. Occasional small cysts are often seen.

Microscopically, both epithelium and stroma are involved, but almost invariably the epithelium in a nodule with a predominantly hyperplastic stroma is low and inactive (Fig. 12). Rarely there may be epithelial hyperplasia. This finding agrees with that of Moore (11). Occasionally transitional or squamoid epithelium may be seen in these nodules (Fig. 13). The stroma is loose and pale staining, resembling the periacinar stroma of the breast and has many large plump fibroblasts lying between the fine stromal fibres. It is distinctly separated from the dense eosinophilic collagen framework of the prostate but stains selectively with the usual collagen stains. Occasionally there are a few included strands of smooth muscle. The stromal hyperplasia

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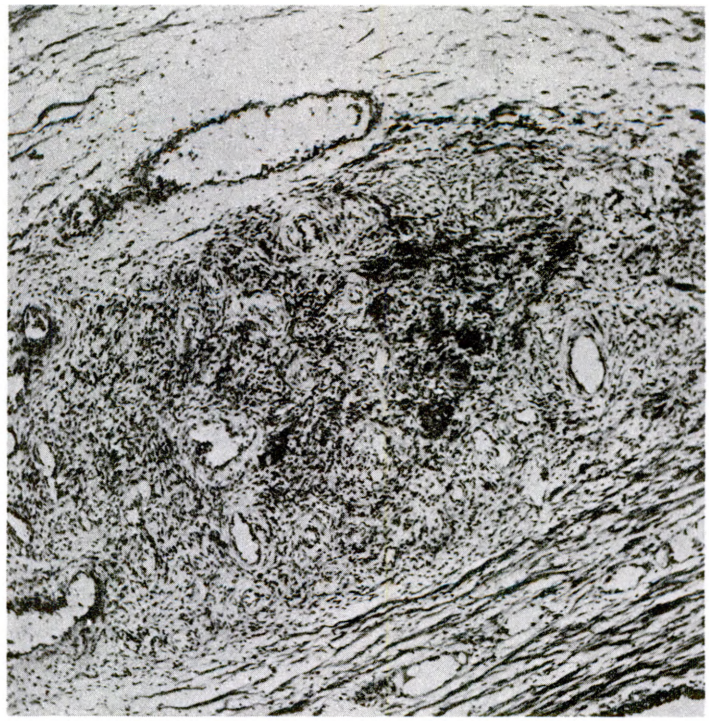

FIGURE 11. A small fibromuscular nodule. Well formed blood vessels and strands of plain muscle (dark) are included in the nodule. The margin is ill-defined. (X 65)

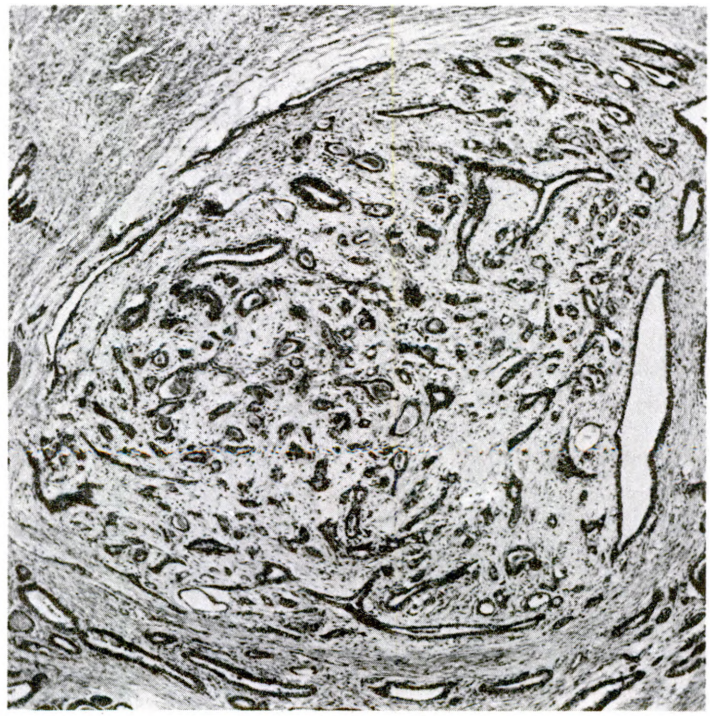

FIGURE 12. Fibroadenomatous nodule. The epithelium is low and inactive. (X 65)

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FIGURE 13. Squamoid epithelium in a fibroadenomatous nodule. (X 65)

may be concentric, producing on section a picture of many small acini lying in collagen (pericanalicular) (Fig. 14), or eccentric and projecting into the dilated often cystic lumen of a duct or acinus (intracanalicular) where it may form an intraductal papilloma (Fig. 15).

The fibromyoadenomatous nodule

This is the common type of large nodule with a varying proportion of each element but predominantly epithelial in most cases. The greater part of the surgically removed "prostate" is of fibromyoadenomatous type as a rule. The naked eye appearances depend on the relative amounts of the various elements. There are commonly the glistening interlacing grey strands of muscle projecting slightly, with yellowish epithelial elements lying between them. Cysts, containing a thin milky fluid or rather thick greyish yellow material and small areas of haemorrhage, are not uncommon.

There is considerable variation in size, the largest I have seen (a surgically removed specimen) measuring almost 15cm. in diameter, others measuring 0.2cm. or less.

Microscopically, the structure varies. The stroma is made up of interlacing strands of smooth muscle and collagen arranged in much the same way as in the normal prostate, but elastic fibres are generally absent. The glandular elements however may be of a number of different types. Commonly there are groups of small or large acini, some of which may be cystic, in which there are often many papillary infoldings (intraacinar and intracystic papillary hyperplasia) (Fig. 16). The structure in these areas may resemble that of the actively secreting female cervix. The epithelium is generally made up of tall columnar cells with pale staining granular cytoplasm, basal vesicular nuclei and rather indefinite luminal margins (Fig. 17), and there is often an inconspicuous basal layer of flattened cells beneath the surface epithelium (Fig. 18) resembling that seen in the normal prostate. Less commonly, the basal layer may be prominent and hyperchromatic, and in some areas solid buds of these cells appear to be growing out into the surrounding stroma. A group of acini showing this change is illustrated in Fig. 19. The hyperchromatic basal cells appear as dark lines surrounding the acini. This can be seen at a higher magnification in Fig. 20, which also shows solid buds of basal cells.

In other areas the hyperplasia may be of small acinar type (Fig. 21), but the acini are lined by two or more layers of darker cuboidal cells (Fig. 22). Areas of rather more irregular small acinar

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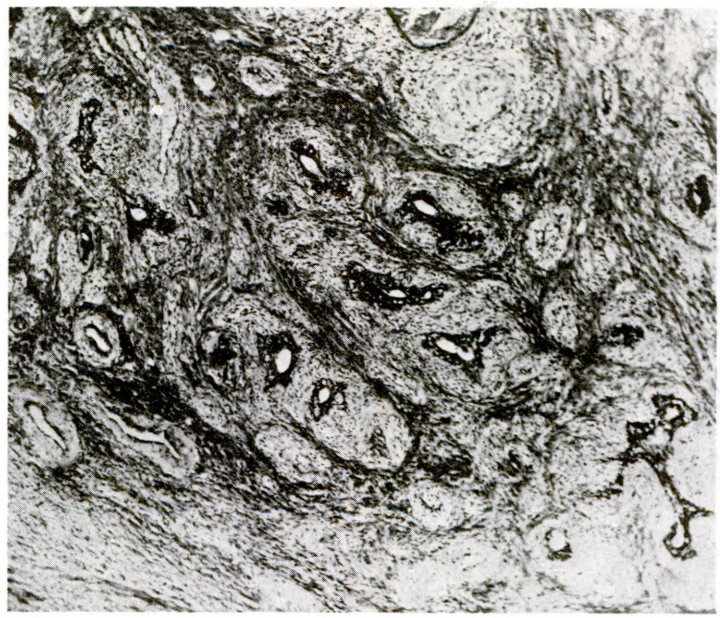

FIGURE 14. Pericanalicular hyperplasia in a fibroadenomatous nodule. (X 50)

hyperplasia are often seen at the margins of large nodules (Figs. 23 & 24). Less commonly the epithelium may be arranged in cribriform or solid trabecular patterns (Figs. 25 & 26).

Transitional epithelium may be found in many nodules (Figs. 27 & 28), and sometimes buds of proliferating epithelium of this type may show a spindle cell metaplasia with the cells growing out into the stroma. Occasionally cells of this type may grow along the acini which may be filled with solid masses of these cells. This intraacinar spindle cell proliferation is shown in Figs. 29 & 30.

FIGURE 15. Intracanalicular hyperplasia in a fibroadenomatous nodule. (X 100)

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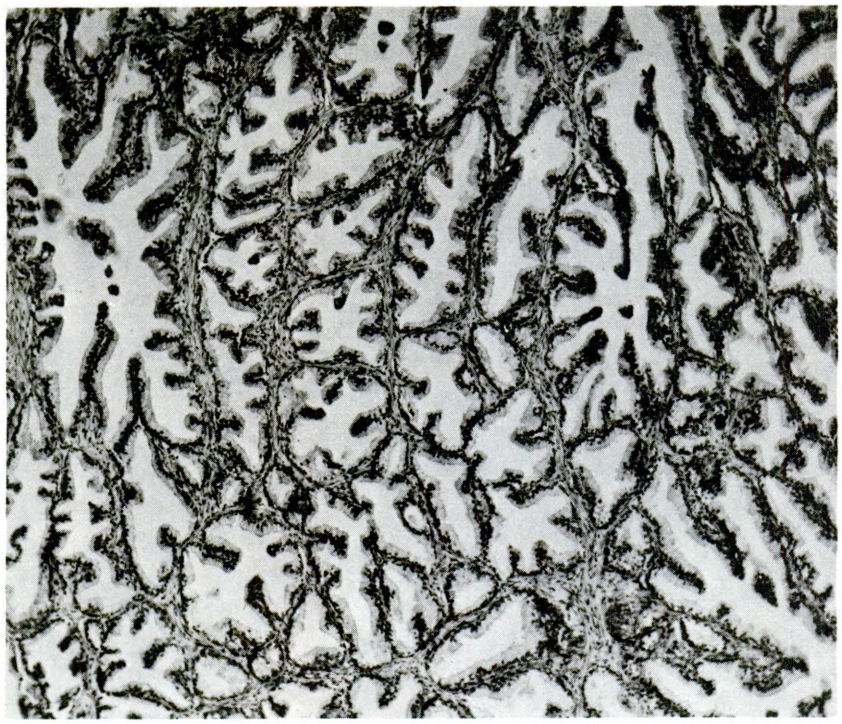

FIGURE 16. An area of intraacinar papillary hyperplasia. The structure resembles that the female cervis. (X 65)

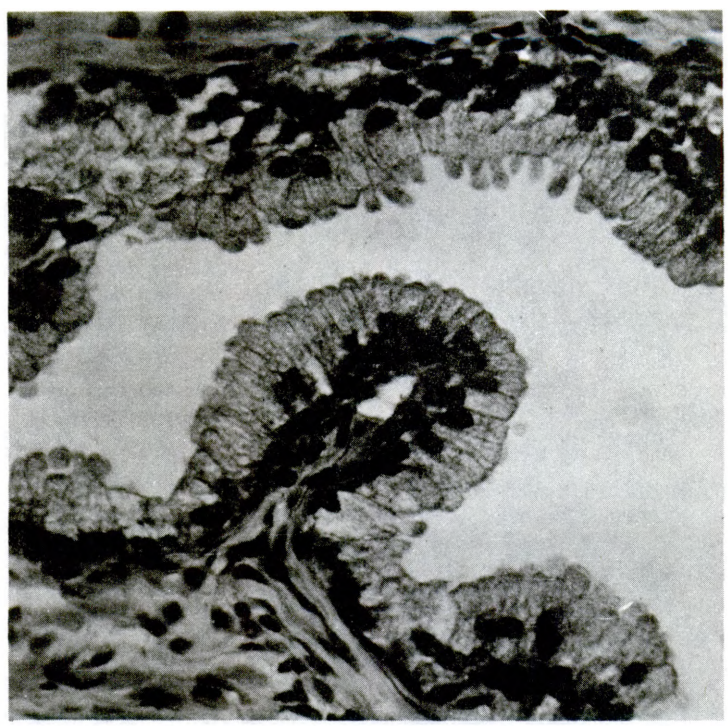

FIGURE 17. Intraacinar papillary hyperplasia showing tall columnar epithelium with pale staining finely granular cytoplasm and rather indefinite luminal margins. The nuclei are basal. (X 540)

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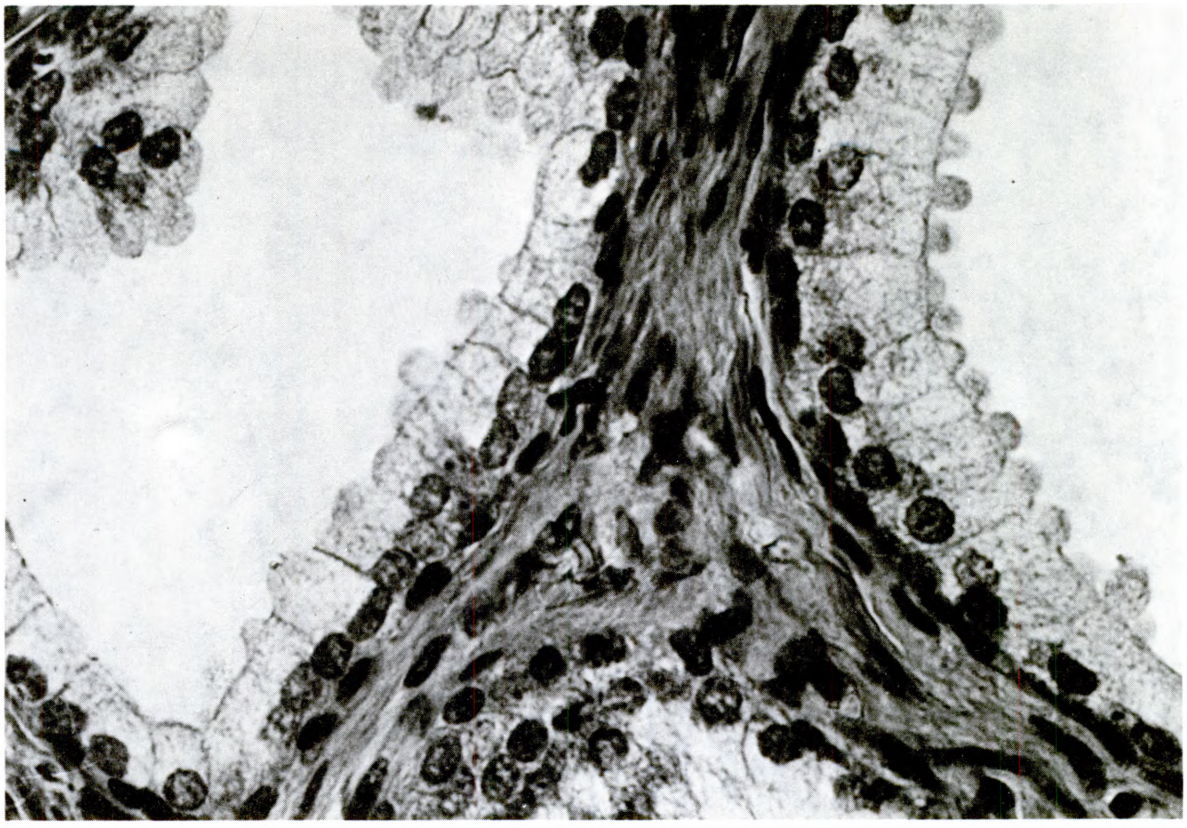

FIGURE 18. The epithelium in intraacinar papillary hyperplasia showing the inconspicuous layer of flattened basal cells beneath the surface epithelium. (X 870)

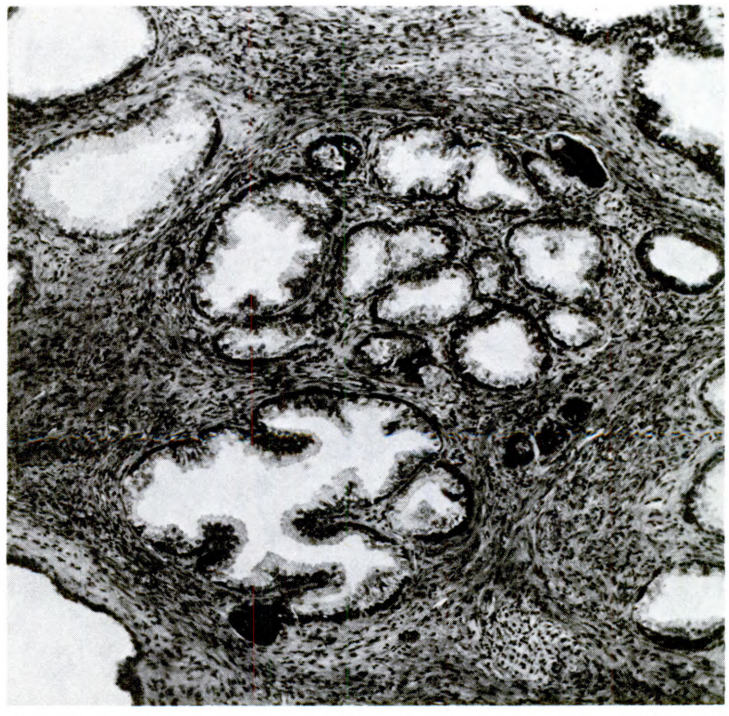

FIGURE 19. A group of acini with a prominent layer of basal cells which appear as dark lines surrounding the acini. (X 100)

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FIGURE 20. Hyperchromatic basal cells beneath the surface epithelium between two solid buds of basal cells apparently growing into a stroma. (X 870)

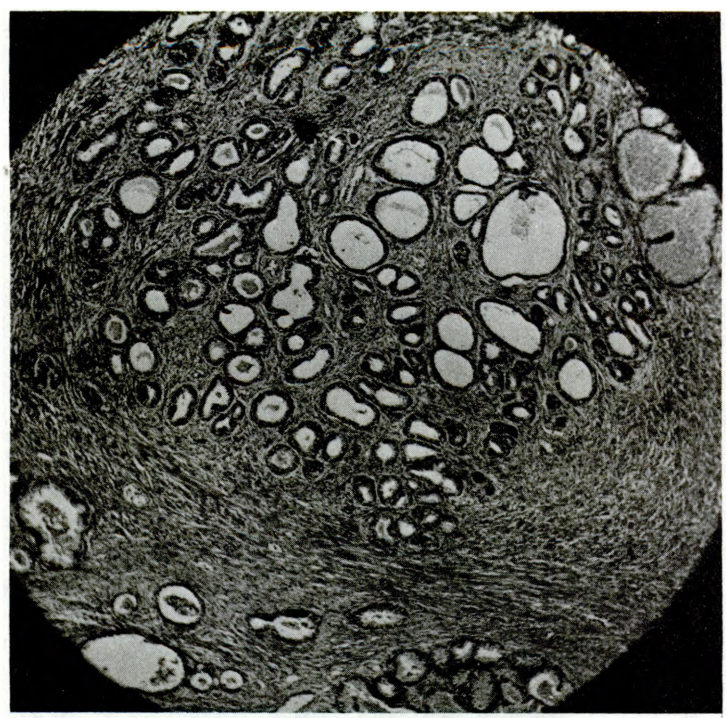

FIGURE 21. Small acinar hyperplasia in a fibromyoadenomatous nodule. (X 40)

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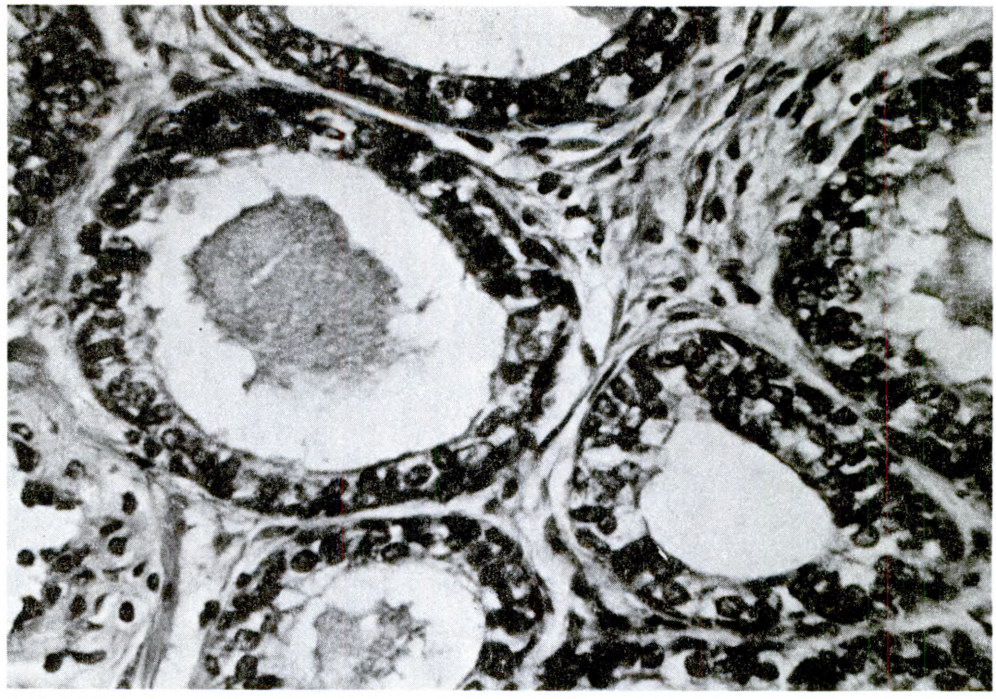

FIGURE 22. Small acinar hyperplasia. The acini are lined by two or more layers of cuboidal cells. (X 540)

Other changes in the nodules

Simple atrophic changes are common in the fibromyoepithelial nodules and are similar to those seen in the outer prostate. Cystic change is particularly frequent, the cysts and atrophic acini being lined by flattened low cuboidal epithelium.

Corpora amylacea are often seen in acini of the nodules, and small calculi may be found in cysts lying between the nodules and the outer prostate. There is often a chronic inflammatory reaction around these cysts.

Recent infarcts are common and if the whole gland is examined can often be seen to involve the urethra (Fig. 31). Later there is peripheral fibrosis in which islands of metaplastic "squamoid" epithelium is found (Fig. 32). Scar tissue with included irregular metaplastic epithelium probably represents the end result of an old infarct (Fig. 33). Areas of prostatic cancer, usually extending from the posterior of the gland (Fig. 34), were found in about 10% of all patients. The incidence is directly related to the number of sections examined.

Inflammatory changes in the nodules and surrounding tissues

Acute or chronic inflammatory changes are present in many nodules, particularly the fibromyoadenomatous type, and these may be focal or diffuse. Of the focal lesions, large or small, acute or chronic abscesses may be found, often associated with a diffuse purulent or lymphocytic infiltration of the surrounding tissues of the nodule.

Many chronic inflammatory lesions in the nodules are associated with distended ducts or acini, which are filled with foamy secretion (probably fatty) or granular basophilic debris. These ducts may rupture into the stroma. Sometimes the whole wall of a duct or acinus becomes necrotic and infiltrated with polymorphs. In either case the contents are released into the surrounding stroma. This is followed by an inflammatory reaction, the type probably depending on the nature of the duct or acinar contents. The reaction may be purulent or more commonly of a chronic granulomatous type. Less commonly there may be hyaline degeneration of part of the wall of a duct or acinus with round cell infiltration only around the affected area.

Occasionally, small localized granulomatous "tubercles" may develop around inspissated secretion in small acini. These lesions may contain "epithelioid" cells and giant cells which may be of foreign body type or derived from damaged epithelium.

Small areas of hyaline focal necrosis with round cell and histiocytic reaction in the surrounding tis-

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FIGURE 23. An area of rather irregular small acinar hyperplasia at the margin of a large fibromyoadenomatous nodule. (X 65)

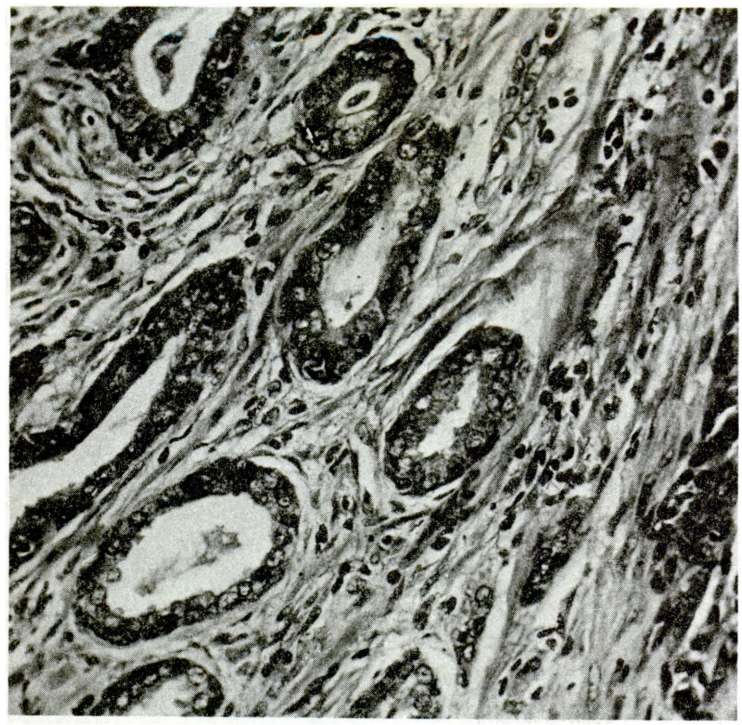

FIGURE 24. A higher power view of an area shown in Fig. 46. (X 340)

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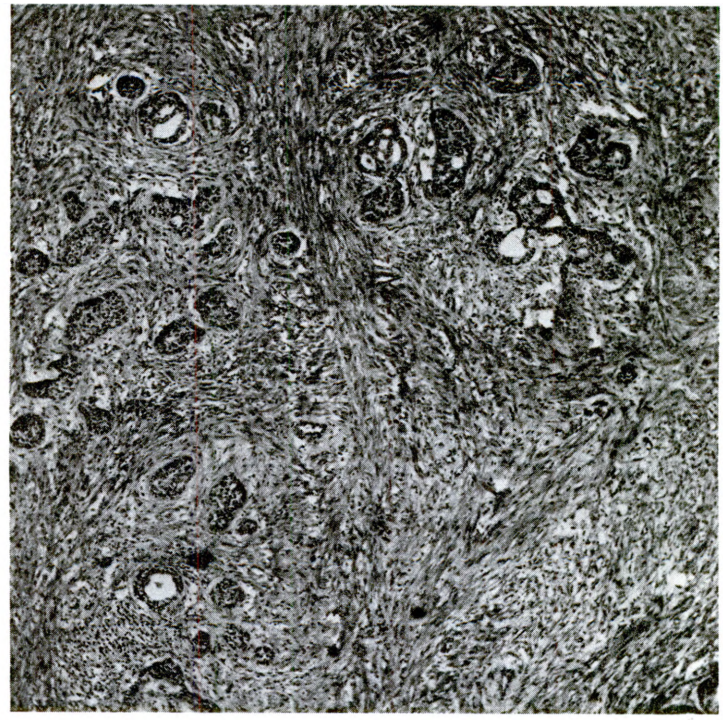

FIGURE 25. Showing solid trabecular and cribriform arrangement of epithelium in a fibromyoadenomatous nodule. (X 65)

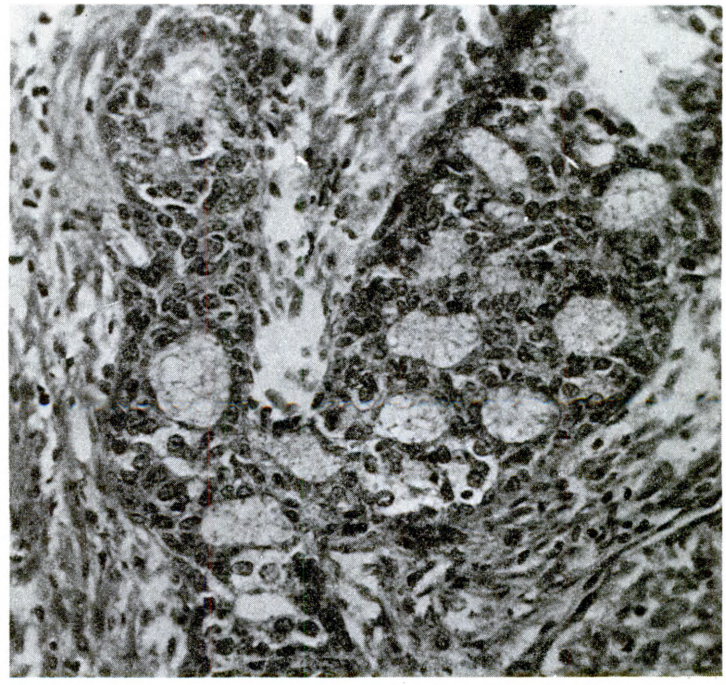

FIGURE 26. Cribriform arrangement of epithelium in a fibromyoadenomatous nodule. (X 330)

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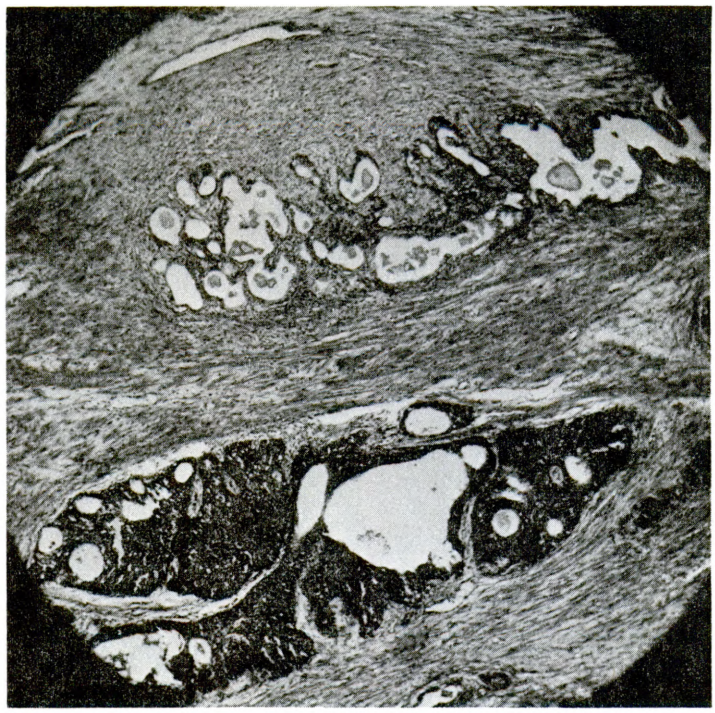

FIGURE 27. Transitional cell epithelium at the edge of a fibromyoadenomatous nodule. (X 40)

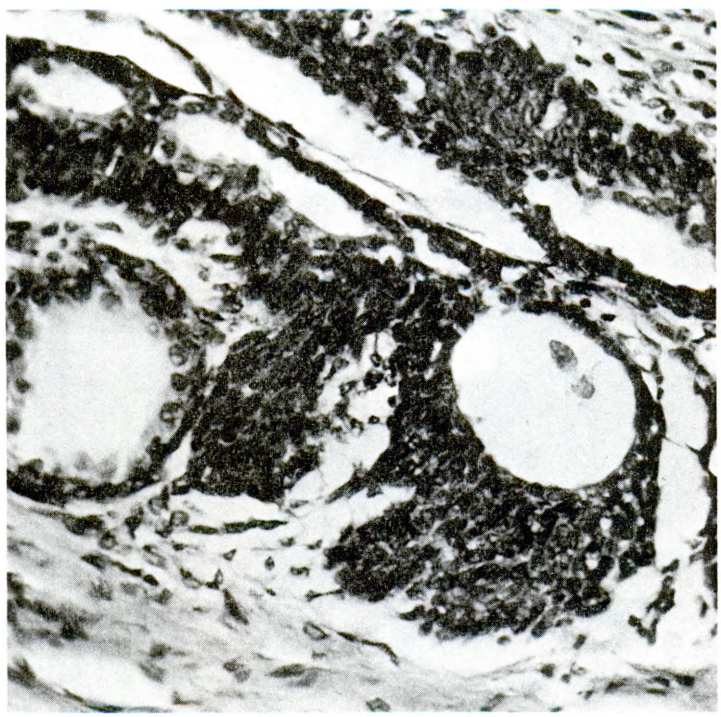

FIGURE 28. Transitional cell epithelium in a fibromyoadenomatous nodule. (X 340)

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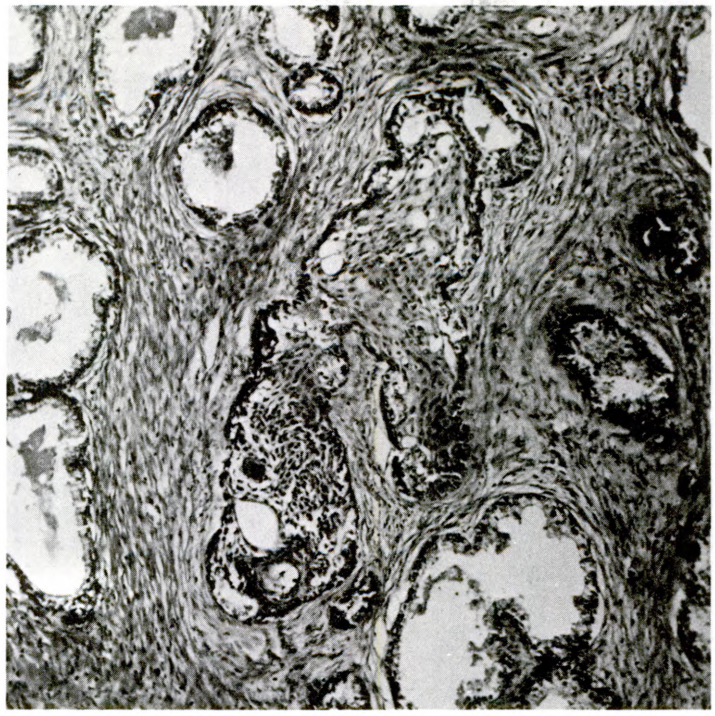

FIGURE 29. Intraacinar spindle-cell proliferation in a fibromyoadenomatous nodule. (X 120)

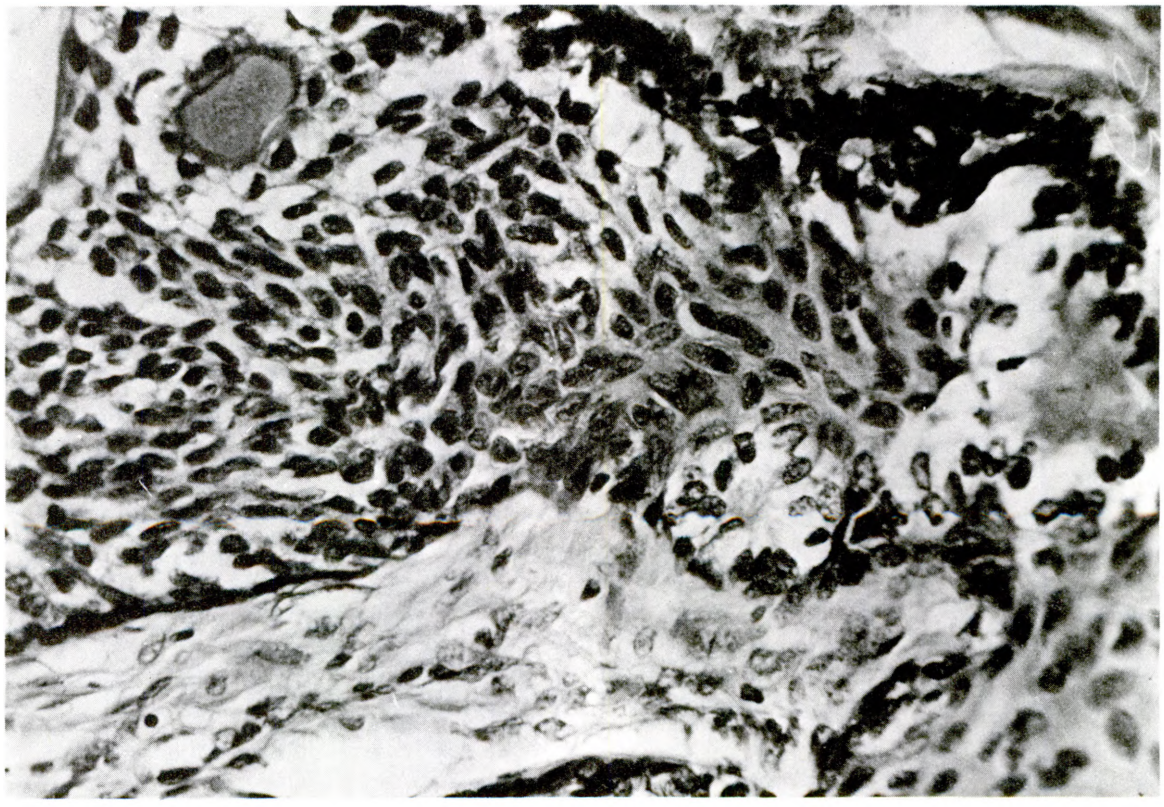

FIGURE 30. A higher magnification showing intraacinar spindle-cell proliferation. (X 540)

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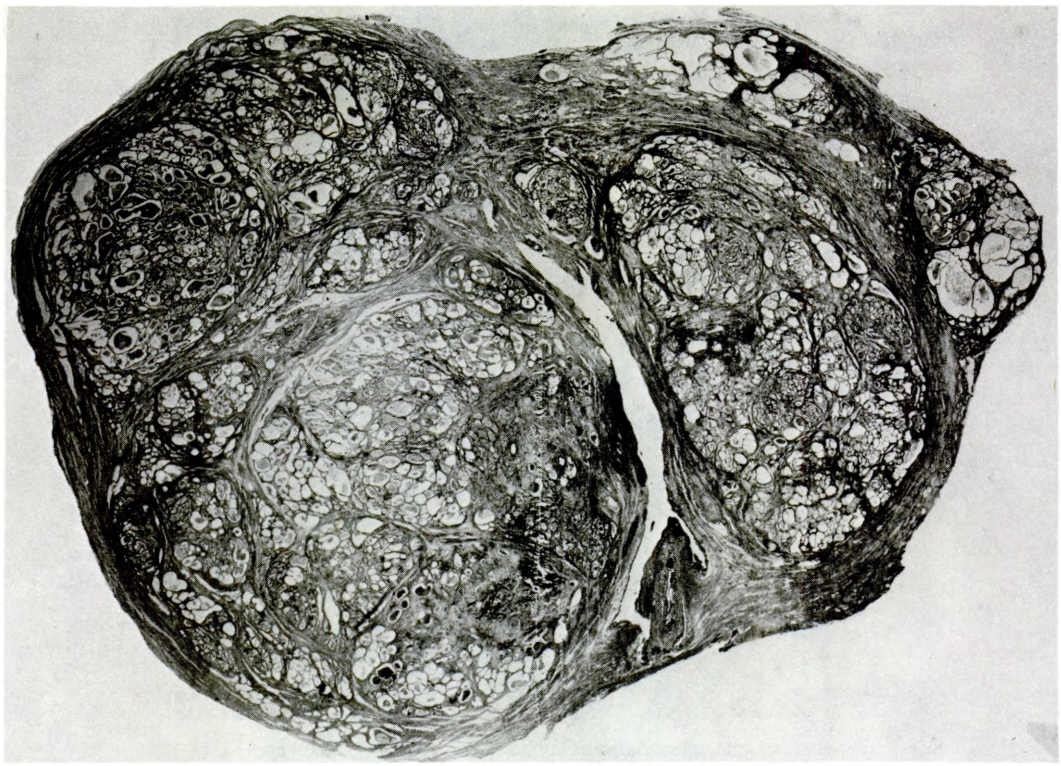

FIGURE 31. Whole section showing benign nodules, utricle (below centre) and a small rim of outer prostatic. There is a small recent infarct, with a slough (dark) over the overlying urethral ulcer, on the left. (X 3)

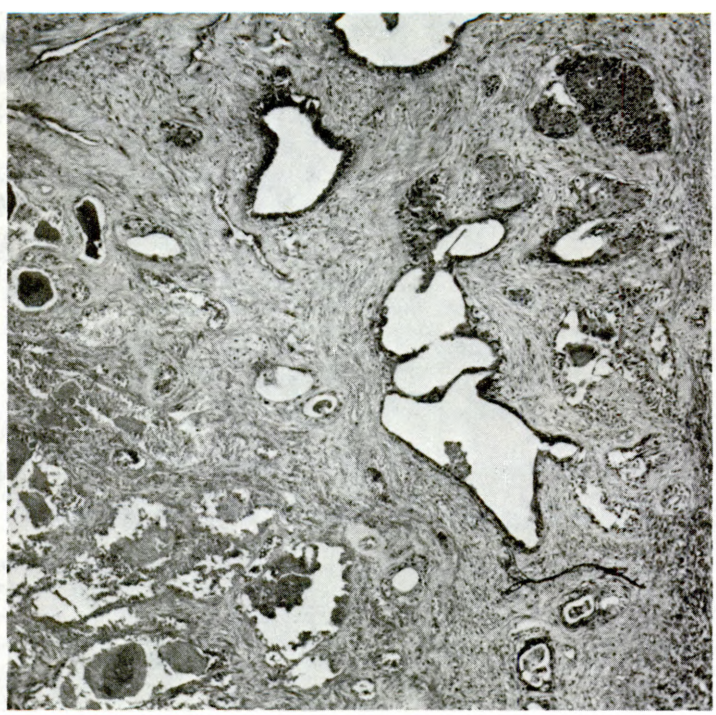

FIGURE 32. The edge of a recent infarct (bottom left) with metaplastic epithelium (top right) in scar tissue. (X 65)

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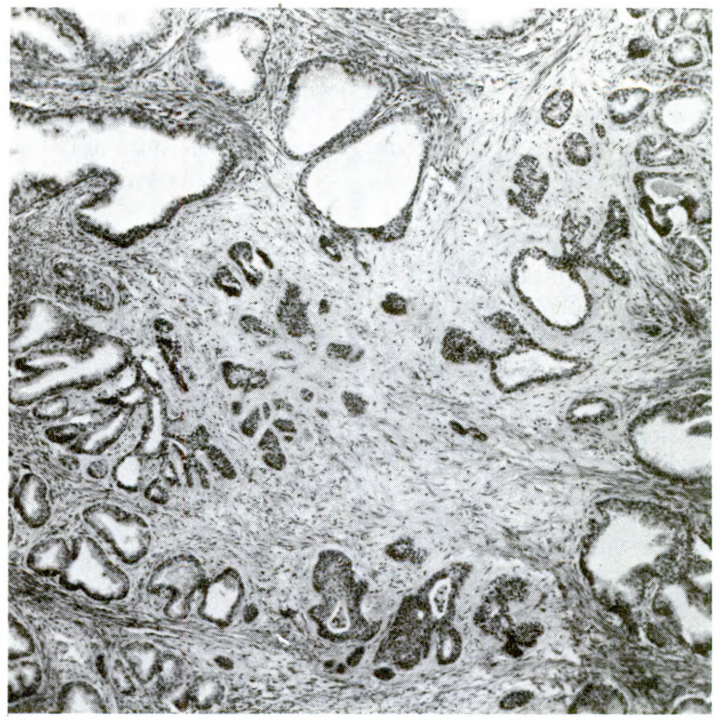

FIGURE 33. Scar tissue with included metaplastic epithelium, probably an old infarct. (X 65)

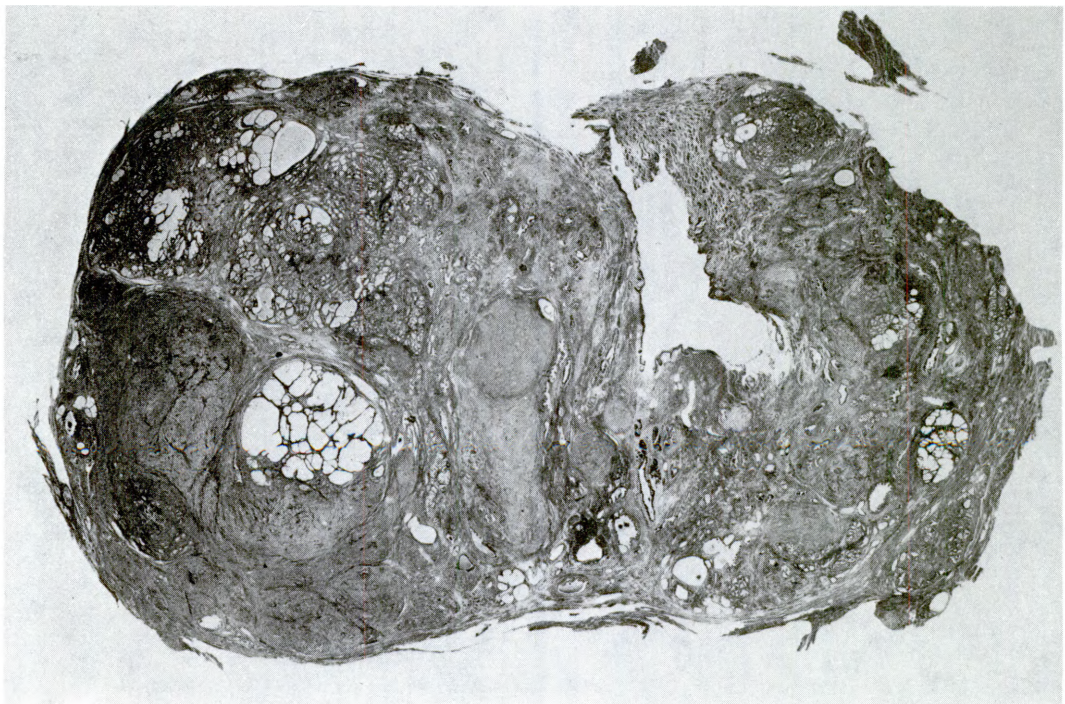

FIGURE 34. Carcinomatous tissue (dark, homogeneous) at left postero-lateral angle partly replacing benign nodules. There is a small fragment of tumour on the extreme right. (X 3)

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sues may be found in the nodules or in the periurethral tissues. A diffuse type of chronic inflammation is common in the periurethral area, in the tissues between the nodules and in the nodules themselves, particularly at their margins. This diffuse lesion often retains a nodular character (Fig. 35) which is probably due to the anatomical arrangement of the periductal and periacinar muscle. These diffuse lesions closely resemble the lesions of "granulomatous prostatitis" (13, 14 & 15). In this condition there is a nodular granulomatous reaction with giant and epithelioid cell follicles which develop around ducts and acini and ultimately fibrose. It is possible that this disease is a more marked form of the chronic inflammatory lesions just described. These latter lesions also may heal by fibrosis leaving scars which sometimes contain small islands of metaplastic epithelium.

Ultrastructure

The ultrastructure of the normal and benign hyperplastic gland has been described by several workers (16). Appearances reflect those seen under the light microscope. The acini are lined by columnar cells, usually in a single layer, with occasional more homogeneous basally situated "myoepithelial" or "reserve" cells lying on the epithelial side of the basement membrane (Fig. 36). The columnar cells vary in height depending on the degree of secretory activity. They contain the usual cell constituents. In the taller cells the Golgi complex is prominent, and there are primary lysosomes and many secretory vacuoles mostly in the luminal zone. The vacuoles contain electron dense granules or small membrane-bound vesicles (Fig. 37). Small sac-like bulges are often present on the luminal surface (Fig. 37). Similar structures have been described and illustrated in the mouse prostate after testosterone stimulation (17). The bulges become progressively larger and finally rupture to release the prostatic secretion. In other areas there are many microvilli on the luminal surface (Fig. 37).

Endoplasmic reticulum is scarce, but there are

FIGURE 35. Granulomatous reaction in the outer prostate. The nodular character of the lesion is due to the anatomical arrangement of the periacinar muscle bundles.

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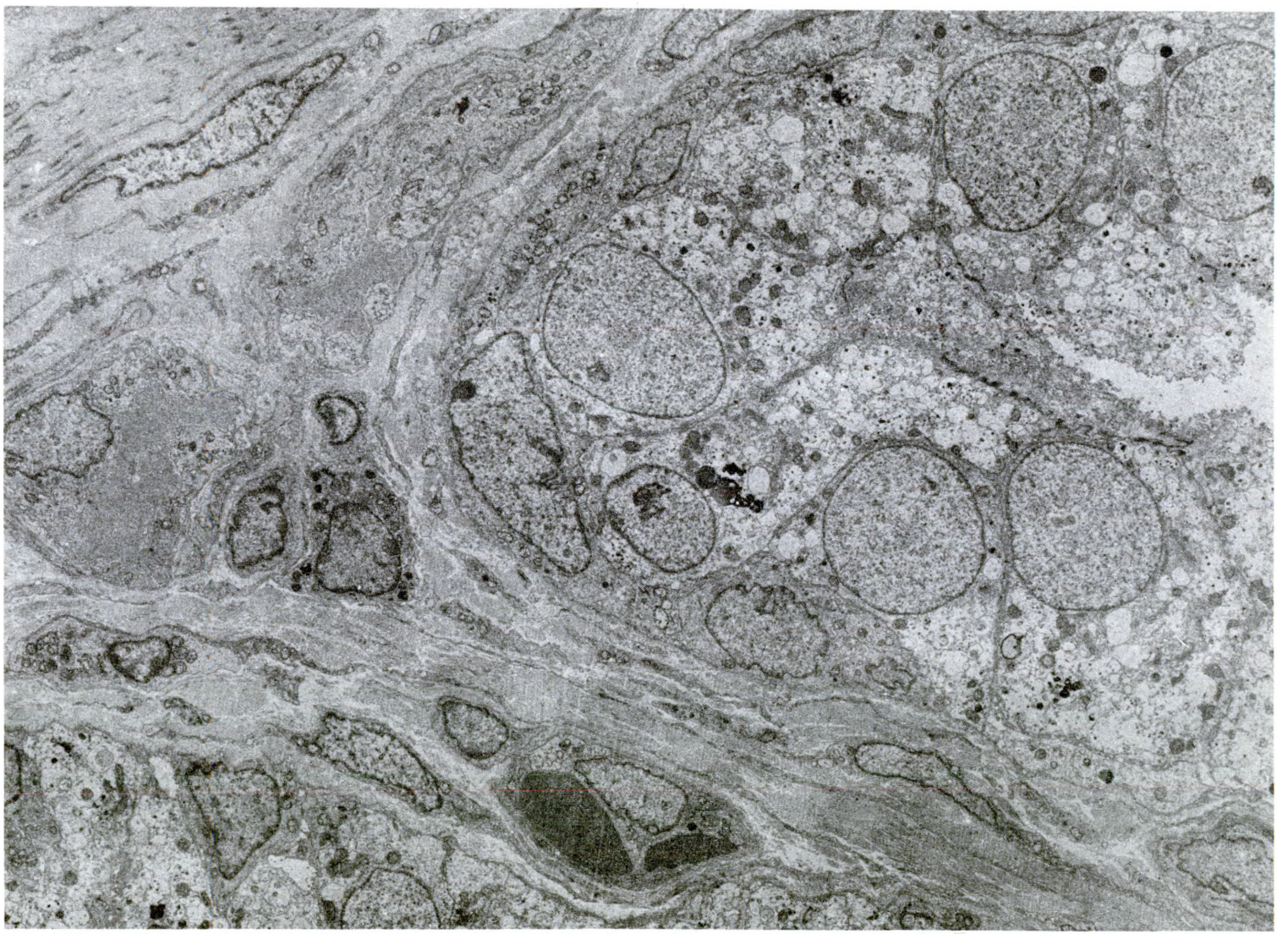

FIGURE 36. Acinus with clear columnar epithelial cells and denser "myoepithelial" cells. Processes of sub-epithelial basal cells and capillaries can be seen in the stroma. (X 3,800)

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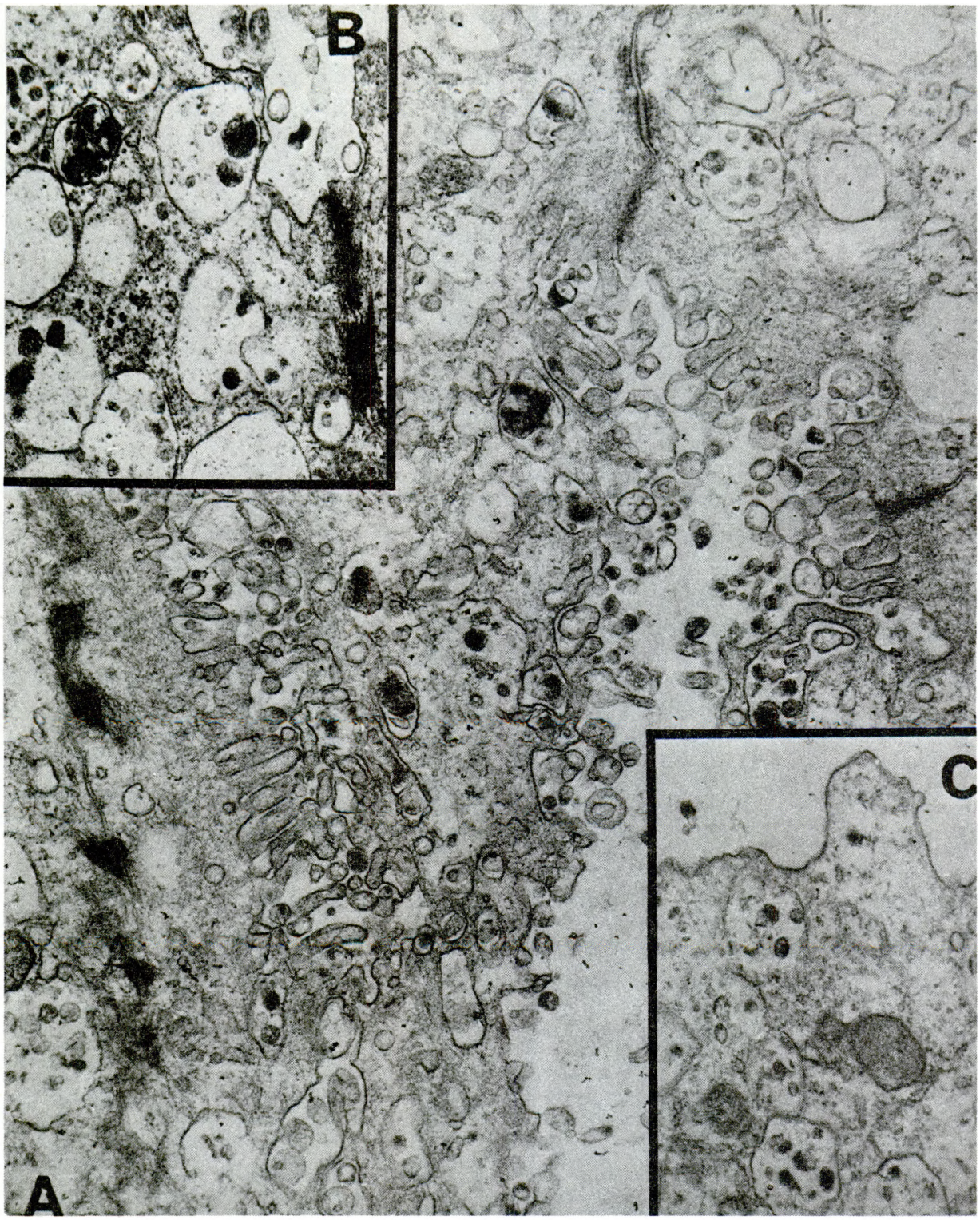

FIGURE 37. Luminal portions of secretory cells:

a. many microvilli

b. secretory vacuoles, some empty, some containing vesicles

c. small luminal bulge. (X 30,000)

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many free ribosomes. Many cells contain numerous fine fibrils scattered throughout the cytoplasm. Junctional complexes are present near the luminal border and between the epithelial cells and the myo-epithelial cells (Figs. 37, 38). There are often small triangular spaces between the epithelial cells at their basal ends. The "myo-epithelial cells are triangular or flattened and have large and sometimes irregularly convoluted nuclei. There is a small Golgi zone, with scattered mitochondria and free ribosomes and often many fine cytoplasmic filaments. The processes of these cells may extend for some distance and make specialised contacts with neighbouring "myo-epithelial" cells (Fig. 38). Mao and Angrist (18) have described these cells in detail and believe that they differ from myo-epithelial cells in other organs. The basal lamina is usually thin and well defined, but reduplication and great thickening may be found in some areas (19). Hemidesmosomes are common particularly under the myo-epithelial cells (Fig. 38). In most cases a narrow zone of collagen and elastic tissue separate the basal lamina from the long processes of the sub-epithelial basal cells. These mesenchymal cells resemble those seen in the mouse (17) and rat prostate. Their function is not known.

The ultrastructure of the stromal and fibromuscular nodules reflect the cellular pattern seen under the light microscope. In fibromuscular nodules the mixed cell population of fibroblasts and smooth muscle cells arranged around small blood vessels is clearly seen (Figs. 39, 40).

Structural features influencing symptoms and treatment

The predominant symptom of benign enlargement is urinary obstruction, but it must be remembered that this is not, as a rule, only due to the presence of benign enlargement, which is commonly found at autopsy in patients without any clinical evidence of urological disease. Although benign nodular hyperplasia alone may cause urinary obstruction either by physically obstructing the urethra or by interference with the muscle or nerves supplying the sphincter this is relatively uncommon. If tissue removed at operation from the prostate is examined, some superadded condition, often vascular, is almost invariably found. Infarcts are common, and there may be acute or chronic inflammation or not infrequently quite large and unsuspected areas of carcinoma. Thus the symptoms of benign nodular hyperplasia may be produced by a wide variety of lesions, some of which may be transitory. This makes it extremely difficult to assess the results of treatment in the short term; any improvement may not be a direct consequence of the treatment used. This may help to explain some apparently contradictory results. Good and bad results have been described following almost every type of endocrine treatment.

In the normal experimental animal the epithelium, muscle and connective tissue respond in a standard and predictable way to hormones. Androgen depletion, for example, causes atrophy of the whole gland and androgen administration will reverse this atrophy and stimulate secretion by the prostatic epithelium. Similarly the effects produced by other natural or synthetic steroids can be well defined. However, the test animals used are young and healthy, and the cellular response of all elements of the gland is uniform since all are in a normal responsive state. In the ageing human male the wide range of patterns found which may range from the actively secretory to the completely atrophic in a single microscope field, indicates that the cellular response in the prostatic epithelium and stroma in these patients is grossly abnormal. In view of the wide range of structures present in the enlarged gland, it seems unlikely that any one drug can produce a sufficient degree of atrophy of all the different types of epithelium, connective tissue and muscle to provide a permanent cure.

Summary and Conclusions

The gross and microscopic anatomy and ultrastructure of benign nodular hyperplasia is described. The condition arises from the inner group of glands, as a result of hyperplasia of cell elements—epithelium, muscle and stroma. The nodules contain varying proportions of each cell type. Clinical symptoms are rarely due to benign nodular hyperplasia alone and are usually precipitated by some additional lesion such as an infarct or infection.

Acknowledgement: Valuable technical assistance given by A. W. Carbonell, Esq.

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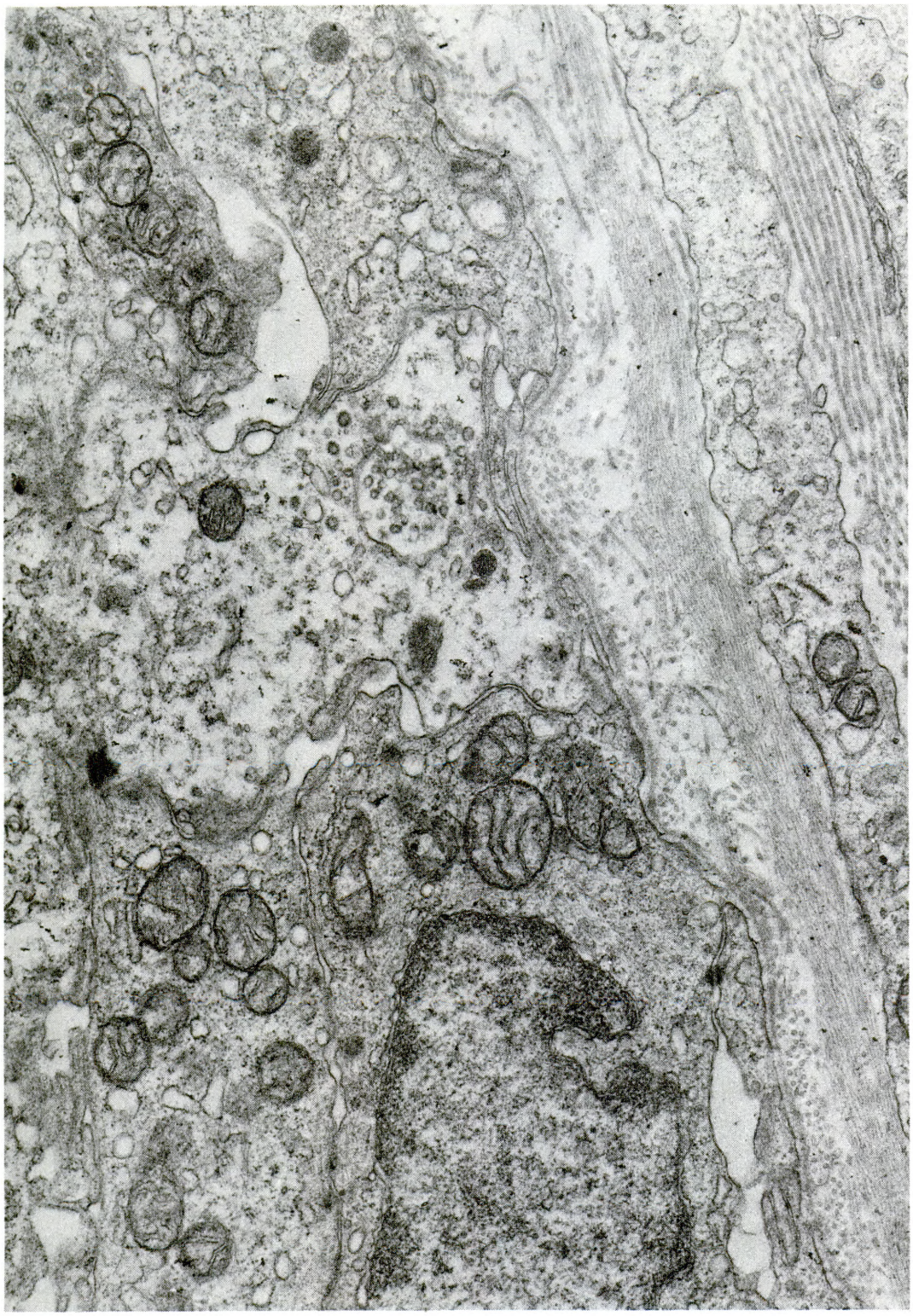

FIGURE 38. Basal area of epithelium. Part of the myoepithelial cell (bottom left) showing a specialised junction with the process of a neighbouring "myoepithelial" cell. The adjoining secretory cell contains a vesicle filled vacuole. Basal lamina, sub-epithelial collagen and elastic layers and the process of a sub-epithelial basal cell can be seen on the right. (X 30,000).

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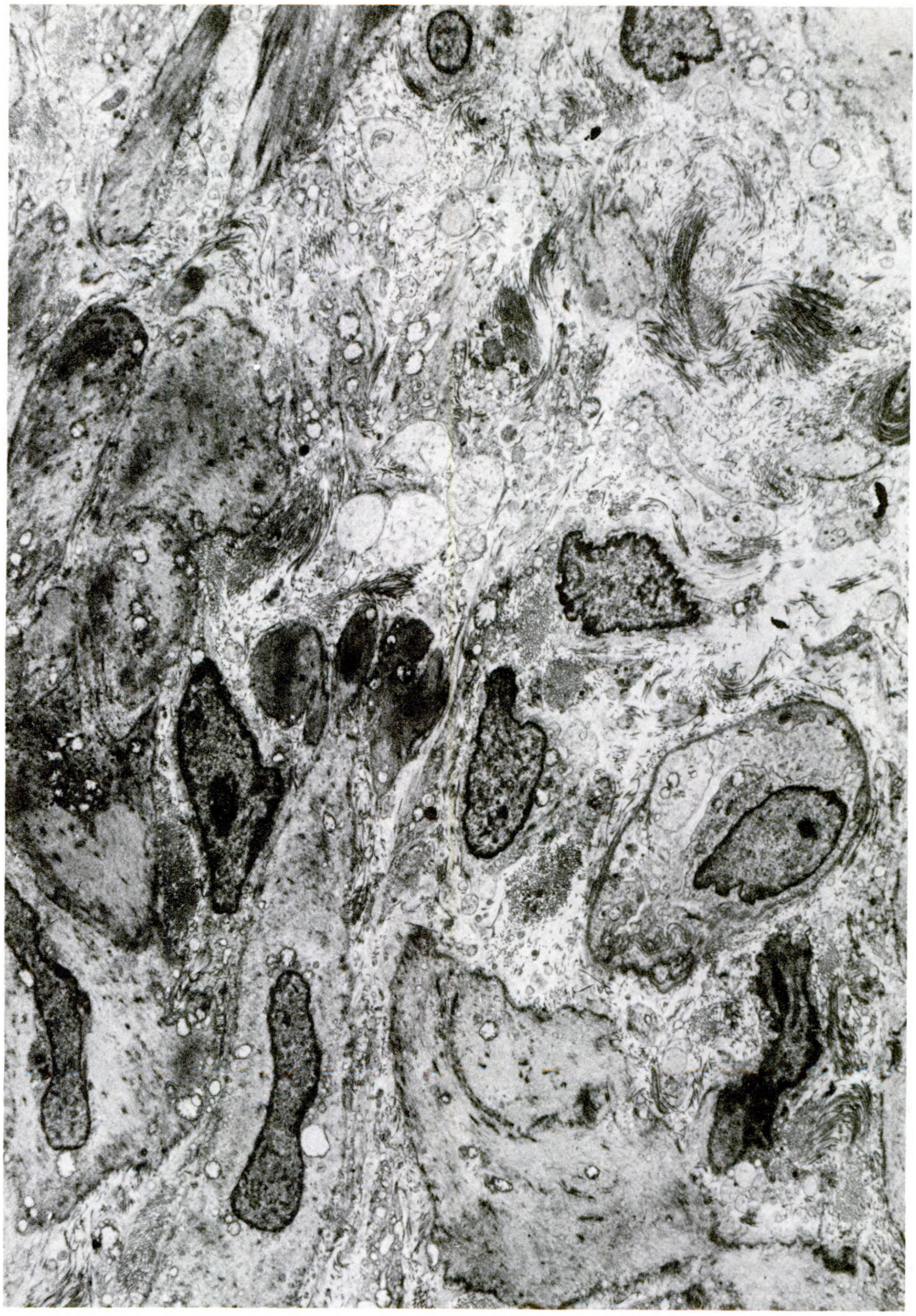

FIGURE 39. Edge of a fibromuscular nodules. (X 5,000)

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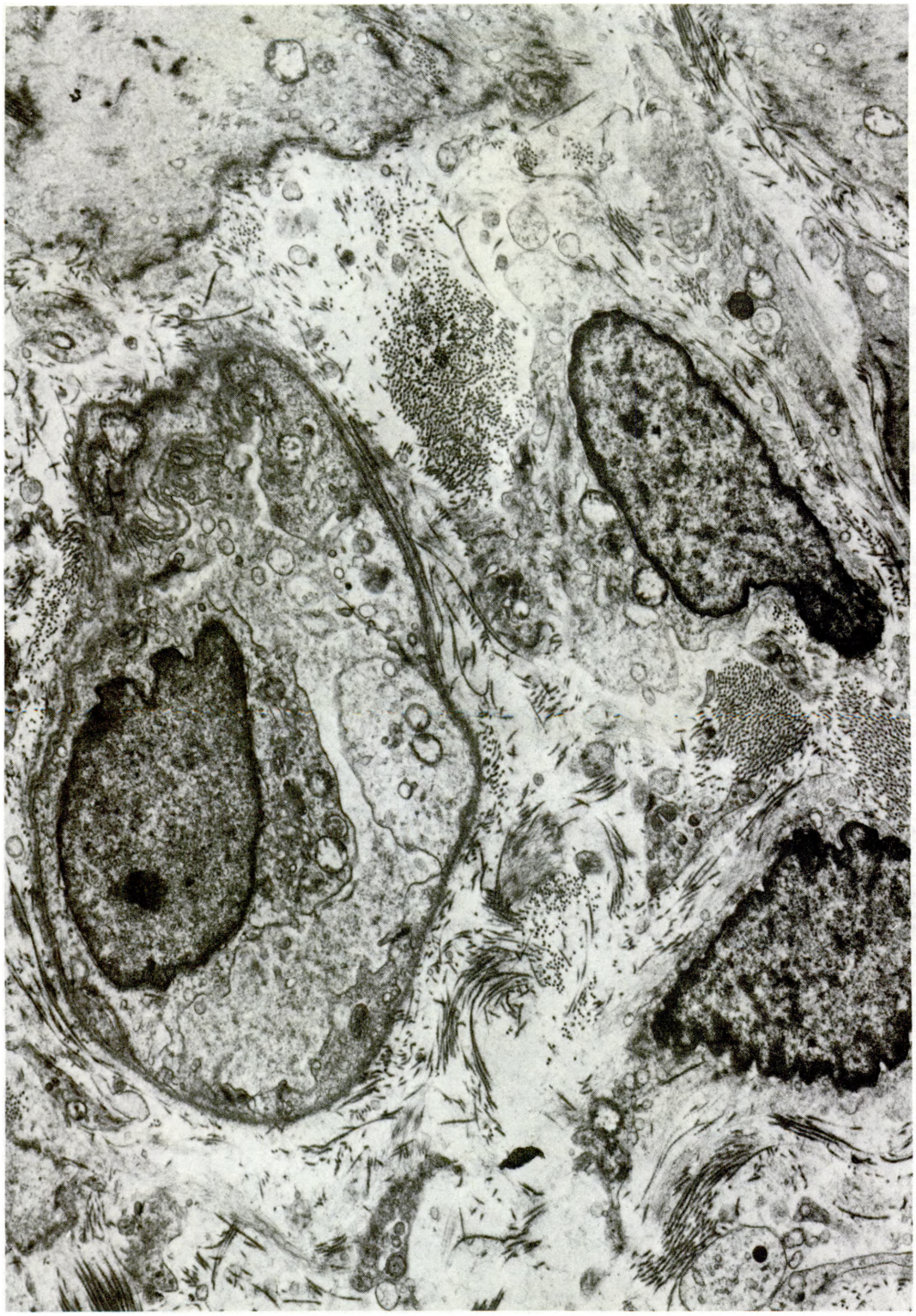

FIGURE 40. A higher power showing blood vessels, fibroblasts and muscle cells. (X 12,000).

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DISCUSSION

M. TANNENBAUM: Dr. Franks, you have done a lot of electron microscopy, and there is a decided interest in the room about the prostate being an immune privileged site. Have you seen any lymphatics in the specimens examined by you under the electron microscope? Are there more or fewer lymphatics than one would expect to find elsewhere?

L. M. FRANKS: I don't think so. I find it very difficult to distinguish the lymphatics from small blood vessels.

M. TANNENBAUM: A number of years ago you stated, as did Dr. Sheldon Sommers, that prostatic carcinoma arises in the atrophic glands, which may be due to local anoxia. Do you still hold to this tenet?

L. M. FRANKS: Yes, actually what I said was that a type of hyperplasia is found in areas where there is atrophy and that proliferation of cells that look like prostatic carcinoma cells can be seen developing from these apparently atrophic areas. Obviously, one can't go further than this.

I. LEAV: Dr. Franks, I was wondering about your comments about myoephithelial cells. Do you really believe that these are true myoepithelial cells? Secondly, as you well know, in differentiated carcinoma of the prostate there is usually a single row of cells rather than a double row. The absence of the layer of "myoepithelial" cells or "reserve" cells is considered a helpful criterion in establishing the diagnosis of malignancy in these instances. I wonder if you would comment on that. Do you really believe there is a myoepithelial cell with contractible abilities or are they progenitor cells of prostatic epithelium?

FRANKS: I don't think one can answer this question. All that one can say is that they look like myoepithelial cells in other organs. I know other people don't agree with this, but they have masses of actin-like filaments and so on. They differ in that they don't have some of the enzymes of myoepithelial cells in the salivary gland and the breast. I don't think any evidence suggests that they are reserve cells. They seem to proliferate outwards, as I showed you in the picture. Now admittedly you could say if they proliferate upward, then you wouldn't recognize them, and that is true. I cannot answer this question satisfactorily.

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METABOLISM AND BINDING OF ANDROGENS IN THE HUMAN PROSTATE GLAND

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Introduction

Based on the classical observations of Hunter, Berthold, Brown-Séquard and Price (see historical review in reference (1), it is known that the growth of the male accessory sexual glands is controlled by the androgenic steroids secreted by the testis. Abnormalities and disorders in the growth pattern of the prostate gland are an invariant feature of the ageing process in the human male and a few species of domestic animals, notably the dog. Some measure of benign prostatic hyperplasia or pathologically related forms of glandular hypertrophy accompanies the onset of senescence in man without exception. Furthermore, ever since detailed medical records have been compiled, invasive prostatic carcinoma has remained the third most common cause of death from malignant disease in males of Western societies, despite dramatic changes in diet, living standards, environmental conditions and social habits since the turn of this century. In a sense, these diseases must be considered a somewhat unfortunate corollary to the acquisition of sexual maturity in the male, for long standing evidence has forged a close connection between the androgens and prostatic disorders, both in man (2) and the dog (3). Consistent with this hormonal etiology was the elegant proposal of Huggins and his collaborators (4) to counter prostatic disorders by the administration of androgen antagonists, especially by the synthetic estrogen, stilbestrol [1]. Despite the universal adoption of this clinical treatment, it does have deleterious side effects, and it is now opportune to appraise other potential means of hormonal chemotherapy in the light of recent advances in our knowledge of the mechanism of action of androgens.

A SURVEY OF ANDROGEN METABOLISM AND BINDING

A current view of the mechanism of action of androgens

Perhaps the outstanding feature of contemporary investigations on the mechanism of action of androgens has been the identification and partial characterization of the specific, high affinity binding mechanism for androgens in male accessory sexual glands. This binding mechanism has been termed the androgen receptor system. The innovative studies on the elucidation of the androgen receptor system have been reviewed in detail by King and Mainwaring (5), and a consensus of this early work is presented in Fig. 1. The binding process occurs by means of an interrelated sequence of distinct steps, only some of which have been fully delineated. a) As particularly well reviewed by Westphal (6), the principal androgen, testosterone, is transported in the plasma as a complex with carrier proteins. This function is mediated by the sex steroid-binding β-globulin (SBG) in man but possibly by the corticosteroid-binding α_2-globulin (CBG) in certain other species. b) Testosterone possibly has a facilitated entry into cells by a process strongly intimated in in vitro prostate superfusion studies (7,8) and is subsequently subjected to extensive metabolism. The principal metabolite is

[1] 4,4′-dihydroxy-α,β-diethylstilbene

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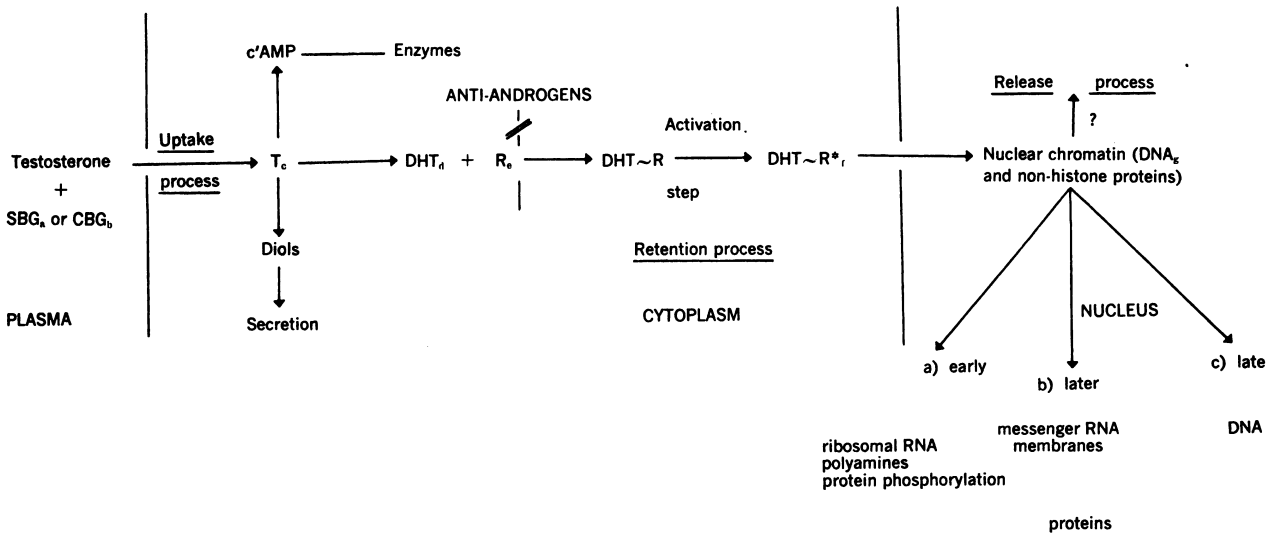
Figure 1. A schematic representation of the mechanism of action of androgens in the prostate gland.



Footnotes a, sex-steroid binding β-globulin; b, corticosteroid binding α-globulin; c, testosterone; d, 5α-dihydrotestosterone; e, cytoplasmic receptor protein; f, activated androgen-receptor complex; g, ultimate destination of receptor complex in DNA and/or non-histone proteins.

5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) (9,10). The various metabolites of testosterone may have distinct physiological roles (5), but 5α-dihydrotestosterone is selectively bound with a high affinity to a cytoplasmic receptor. The resultant complex, after undergoing an activation step which modifies the physicochemical configuration of the protein, is ultimately transported into a restricted number of specific sites in nuclear chromatin. c) The presence of the androgen receptor complex modulates the synthesis of a variety of macromolecular constituents in an integrated sequence of temporal events, ranging from a rapid rise in ribosomal RNA synthesis to late events, such as DNA replication, which occur only after a protracted latent period. The critical importance of the receptor system has been forcibly but admittedly indirectly established by the use of anti-androgens. Two of the more intensively studied anti-androgens, cyproterone acetate [2] and BOMT [3] counter the high affinity binding of 5α-dihydrotestosterone (11,12) and similarly negate the manifestation of the androgenic response in terms of the synthesis of ribosomal RNA (12), proteins (13), messenger RNA (14), polyamines (15) and DNA (16). d) The receptor complex remains in the chromatin for only a finite period of time (17,18), perhaps lasting five or six hours only and then dissociates or is actively broken down. There are certain indications of an exit mechanism for androgens (19,20), but more work is needed to clarify this particular aspect of the overall binding process. In general terms, this mechanism of action is applicable to the accessory sexual glands of all the common experimental animals and to many other androgen-sensitive cells (21).

The mechanism of action presented in Fig. 1 is somewhat superficial, but it suffices to provide a satisfactory basis for discussion on the human prostate gland. In particular, the following criticisms could be levelled at this model: a) It implies almost exclusive prominence to receptor-mediated events; certain biochemical processes are refractory to anti-androgens, especially certain enzymes engaged in pentose sugar metabolism and whose activity may be regulated by the formation of adenosine 3′:5′-cyclic phosphate (15). b) Certain aspects of androgen action do not need to be

[2] 6-chloro-17α-acetoxy-1,2α-methylene-4,6-pregnadien-3,20-dione
[3] 6α-bromo-17α-methyl-17β-hydroxy-4-oxa-5α-androstan-3-one

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triggered by nuclear events, mandatorily requiring DNA as template; the induction of β-glucuronidase can be achieved by essentially extranuclear mechanisms (22). c) The mechanism of action presented in Fig. 1 does not provide an explanation of the subtle differences in the androgenic response of androgen-sensitive cells; for example, alcohol dehydrogenase is induced by androgens only in the kidney of certain strains of mice (23), and accessory sexual secretions contain characteristically unique constituents in different species (24). d) The model does not accommodate the nuclear binding of testosterone reported by Bruchovsky and coworkers (25), and this could be of physiological importance.

Studies on human prostate glands

In virtually all respects, the mechanism of action of androgens presented in Fig. 1 may be applied to the human prostate gland, especially to the normal (non-adenomatous) gland and samples of prostatic carcinomata. The metabolism of androgens in human prostate minces in vitro has been widely investigated and admirably reviewed elsewhere (26). The consistent identification of a particularly active 5α-reductase system [4] ensures that the human gland has a pronounced ability for forming 5α-dihydrotestosterone. The formation of this important metabolite was confirmed under conditions in vivo by administration of tracer doses of tritiated testosterone just prior to surgical enucleation of benign prostatic adenomata (27,28).

The specific and high affinity binding of 5α-dihydrotestosterone has also been widely reported in samples of benign prostatic hyperplasia. Following the innovative study by Hansson and his collaborators (29), a more comprehensive study was conducted by Mainwaring and Milroy (28) on both surgical samples of hyperplasia removed at retropubic prostatectomy and normal prostate gland. Nuclear and cytoplasmic receptor complexes were identified (28), and these bore a remarkable similarity to the androgen receptors described formerly in experimental animals (5). A summary of our study is presented in Table 1. One limitation to this study was that the steroid specificity of the receptors was not rigorously pursued. This was due in part to the method of analysis employed, namely sucrose gradient ultracentrifugation, and to the understandable difficulty of obtaining normal prostate gland in any quantity.

Many other reports on the binding of androgens in human prostate gland have since appeared in the literature, and a summary of current findings is presented in Table 2. The conspicuous feature of this survey is that 5α-dihydrotestosterone-specific receptors were either readily identified or that, conversely, the experimental findings were totally negative. This experimental variation can be attributed to two principal causes; first, the notorious lability of the receptor proteins and second, the heavy contamination of the specimens with the serum protein, SBG. Prostatic hyperplastic nodules are exceedingly difficult to homogenize, and inadequate cell breakage results in a spuriously low release of receptor protein; on the other hand, drastic homogenization will result in receptor degradation. It is also significant that the milder means of analysis used in Table 2, such as gradient ultracentrifugation (28,32) or gel-exclusion chromatography (29,30) afforded better results than dextran-charcoal analysis (33,34), during which labile receptor complexes can be destroyed. The comparative advantages of various means for receptor analysis have been appraised in detail by King and Mainwaring (5). The contamination of human prostate samples with SBG is a ubiquitous problem in such studies. The mandatory structural requirement for the binding of a steroid to SBG is the presence of a 17β-hydroxyl group (6), and, accordingly, 5α-dihydrotestosterone is avidly bound to this serum protein. Distinction can be made between receptor and SBG binding by exploiting differences in their physicochemical properties, such as sedimentation coefficient (28,32) and electrophoretic mobility (35), or by judicious use of certain steroid competitors. Various 5α-androstanediols, for example, displace 5α-dihydrotestosterone from the high affinity binding sites on SBG (6) but not from the androgen receptor proteins (5). In summary, it is concluded that the difficulties encountered in the identification of androgen receptors in benign prostatic hyperplasia are compounded of serum contmination, conditions of homogenization and the means of analysis adopted. Androgen receptors, essentially specific for 5α-dihydrotestosterone are thus considered by the present authors to be a characteristic and invariant feature of the human prostate gland.

Studies on the binding of 5α-dihydrotestosterone in metastatic prostate carcinomata are few; one report, however, suggests a high level of androgen binding in this form of diseased prostate gland (36), even exceeding that in benign prostatic hyper-

[4] NADPH-dependent 3-ketosteroid-5α-oxidoreductase

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TABLE 1—The properties of the androgen receptors in the human prostate gland (28)

Tissue type	Conditions of labeling	Type of receptor studied	Physical properties	Steroid specificity: 5α-dihydrotestosterone [a] = 100
Hyperplasia	*In vitro*, 0°C	Cytoplasmic	8S Absent in serum and muscle	Estradiol-17β, 15 Testosterone, 15 Cortisol, 2 Cyproterone acetate
Hyperplasia	*In vivo*	Cytoplasmic	8S Absent in serum and muscle	Testosterone, 10
Normal	*In vitro*, 0°C	Cytoplasmic	8S Dissociated in KC1 to 4S form Stokes radius 79°A Protein[b]	Testosterone, 10 5β-Dihydrotestosterone, 2 Dehydroepiandrosterone 5α-Androstan-3,17-dione Androstenedione
Normal	*In vitro*, 37°C	Nuclear	4.3S S value constant in varying KC1 Stokes radius 46° Protein[b]	Estradiol-17β, 10 5β-Dihydrotestosterone, 2 Cortisol Cyproterone acetate

[a] measured either by binding of [^{3}H] steroids or by displacement of the binding of [^{3}H] 5α-dihydrotestosterone;
[b] effect of enzymes of narrow substrate specificity

TABLE 2—The identification of androgen receptors in benign prostatic hyperplasia

Reference	Positive identification : total specimens examined	Method of analysis employed
Hansson et al (29)	Not given (100%?)	Sephadex G–100
Geller & Worthman (30)	Not given (100%?)	Sephadex G–50
Giorgi et al (31)		Superfusion
Rusen et al (32)	6/6	Gradients
Mainwaring & Milroy (28)	14/27	Gradients; Sephadex G–200
Steins et al (33)	0/7	Charcoal; agar gel
Mobbs et al (34)	0/14	Charcoal

plasia. This important observation will hopefully be confirmed in the near future.

Androgens and the incidence of prostatic disorders

In view of the absolute dependence of prostatic function and glandular proliferation on circulating levels of androgens, it is pertinent to seek a correlation between the incidence of prostatic disorders with aberrations in the secretion or utilization of androgens during aging. The earlier postulate that prostatic disorders were attributable to androgen deprivation during senescence (37,38) has not been substantiated by more recent investigations. First, it is now evident that the circulating levels of testosterone do not fall dramatically with the onset of age (39), and second, the extent of androgen binding in serum is not profoundly altered during senescence (40). Furthermore, other lines of evidence suggest that disorders in the growth of the prostate gland are promoted by subtle changes in androgenic stimulation (26,41). Consistent with this viewpoint is the very important observation that the concentration of 5α-dihydrotestosterone is particularly elevated in the periurethral region of the human prostate gland where the process of hyperplasia originates (42). In addition, 5α-dihydrotestosterone has proven mitogenic activity (43), and studies with a variety of anti-androgens implicate the 5α-dihydrotestosterone-receptor system in the control of DNA replication in the prostate gland (16). Taken in concert, these diverse observations promote the concept that changes in the utilization of testosterone within the prostate gland itself with age are responsible for inducing abnormalities in prostatic growth.

Based on the mechanism of action proposed earlier in Fig. 1, at least three plausible possibilities exist. First, age-associated defects in the putative uptake mechanism may enable more testosterone to enter the gland. Second, the intracellular receptors

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may be structurally changed with age so that they bind 5α-dihydrotestosterone with an even higher affinity. Third, the exit mechanism may be impaired during senescence, leading to an accumulation of androgenic steroids within the prostate gland. Of these possibilities, the elegant studies conducted by Giorgi and her collaborators (31) do suggest a very high level of retention of androgens in benign prostatic hyperplasia maintained under the conditions of superfusion, in vitro.

Androgens and chemotherapy of prostatic disorders

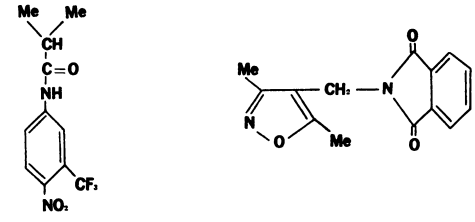
With current advances in surgical techniques, it seems unlikely that chemotherapy directed towards the ablation of the androgen receptor system will play an important role in the treatment of benign prostatic hyperplasia in the foreseeable future. Furthermore, assessment of androgen binding in needle biopsy specimens of the prostate gland are unlikely to provide an improved means of diagnosis. The human prostate gland appears to contain an efficient androgen-binding mechanism both in the normal (non-adenomatous) state and in pathological conditions. This conclusion is in sharp contrast with the situation prevailing in the clinical manipulation of human breast cancer where measurement of estrogen binding can serve as an early indicator of the onset of malignancy and also as a discriminant in the selection of the most appropriate means of chemotherapy (44).

The situation is entirely different in the context of potential chemotherapy of invasive prostatic carcinoma for in this case, surgery is seldom performed and is widely deemed to be hazardous and, at best, undesirable. Three stages of the selective androgen binding mechanism would appear to be particularly amenable to inhibition by chemotherapeutic agents. Potential compounds for suppressing the uptake and retention of androgens are presented in Fig. 2. Anti-androgens capable of competing for the high affinity binding sites within the androgen receptor system should ideally display three characteristics; low toxicity, high biological potency at low concentrations and negligible intrinsic hormonal activity. The non-steroidal anti-androgens, flutamide [5] and DIMP [6], eminently satisfy these exacting criteria (45,46), and it is to be hoped that they will be subjected to close clinical appraisal. The steroid-related compound, 4-androsten-3-one-17β-carboxylic acid, is an inhibitor of the enzyme 5α-reductase (47), but it would seem that its clinical usefulness may be limited by the manifestation of intrinsic androgenic activity during long-term administration due to enzymic modification of the 17β-substituent group. Finally, certain 19-norprogesterone derivatives, such as gestonorone capronate [7], appear to suppress the uptake of testosterone (48), and since this compound also has the additional ability to inhibit 5α-reductase activity, its clinical efficacy in the chemotherapy of prostatic cancer is potentially of the highest order.

Conclusions

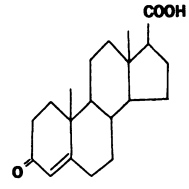
Recent studies conducted in a number of laboratories have led to a remarkable improvement in our understanding of the mechanism of action of androgens, notably in the elucidation of the selec-

Figure 2. Compounds important in future studies.

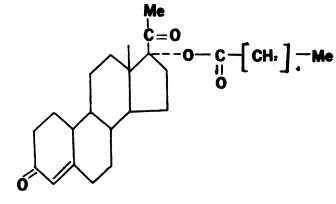


a) Flutamide

b) DIMP



c) 4-Androsten-3-one-17β-carboxylic acid



d) Gestonorone capronate

Footnote a: In all structures Me represents a methyl group

[5] 4′-nitro-3′-trifluoromethylisobutryl anilide

[6] N-(3,5-dimethyl-4-isoxazolylmethyl)phthalimide

[7] 17-hexanoyloxy-19-norpregnen-4-ene-3,20-dione

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tive binding process for a specific metabolite of testosterone, 5α-dihydrotestosterone. Further studies on the androgen receptor mechanism in the human prostate gland may not necessarily prove to be of outstanding clinical relevance, but a more detailed insight into the processes by which androgens enter and leave androgen-sensitive cells may inspire improvements in the chemotherapy of prostatic carcinoma.

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DISCUSSION

J. GELLER: I would like to comment on our current receptor technique that we are using which is different from the one you quoted. With a prostate mince incubation technique with ^{3}H-testosterone, we were able to detect cytosol receptor about 80% of the time in a fraction of cytosol simultaneously desalted and fractionated on a duo-gel column of Sephadex on top of Bio-gel A 1.5M. We used preincubation with cyproterone acetate to identify receptors and showed that since cyproterone acetate has no effect on steroid binding to TeBG in our system any inhibition in receptor-steroid protein peak with cyproterone acetate would be specific for receptor and not TeBG. It takes about 3–4 hours for our entire fractionation procedure, which gets around some of the problems of the lability of the receptor.

W. I. P. MAINWARING: This is a very useful experimental approach, and the saving of time is important.

N. BRUCHOVSKY: Dr. Mainwaring, I wonder if you would make a comment on an apparent paradox? According to the model of steroid hormone action, in the presence of normal endogenous levels of steroids most of the receptors are shifted into the nucleus. Why is it that cytoplasmic receptors are still found in tissues when theoretically the receptors should be entirely localized in the nucleus? Secondly, both you and Dr. Mobbs have done analyses on prostates of men who have been orchiectomized. You reported some time ago that the cytoplasmic receptor level drops following orchiectomy. Why would one expect to find receptors in such neoplastic tissues as these?

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MAINWARING: Obviously saturated receptors must exist in the normal man. In our experience receptors cannot be found in animals that are not castrated. One of the things that should be looked at is receptor turnover. As for the second point, the disappearance of the cytoplasmic receptor after castration is in part an experimental artifact. In our hands the cytoplasmic receptor cannot be detected 3 days after castration of the rat. By that time the gland has atrophied and proteolytic activity is high. If you look for receptors in animals castrated for longer periods, which is what Sullivan and Stratt first did, the proteolytic activity disappears, and receptor proteins can be demonstrated. Thus, the disappearance of the receptor after castration is a non event. I think it's an artifact.

P. ROBEL: I would like to comment on androgen receptors in non-castrated subjects. First we have investigated the amount and distribution of receptor in the ventral prostate of normal adult rats at steady state. About 80% of receptor-dihydrotestosterone complexes are present in the nuclei (12 pmoles/mg DNA), and 20% are in the cytoplasm (2.6 pmoles/mg DNA), although most of the receptor sites in the cytoplasm are occupied with endogenous hormone. The situation is similar in the cytosol of human benign prostatic hypertrophy samples. If the 8–10S regions of glycerol gradients prepared with unlabelled cytosol is collected and dihydrotestosterone content measured by radioimmunoassay and if gradients were made in parallel with ^{3}H-dihydrotestosterone labelled samples to evaluate the amount of available receptor sites, it was found that more than 90% of the receptor sites were occupied. This explains why it is difficult to find a receptor in many BPH samples. Moreover, most of the receptor should be in the nuclear fraction.

G. R. PROUT: We have taken needle biopsies of patients with prostatic carcinoma as well as with BPH. Histological examinations were performed on half of the mirror image of the tissue and the other half was incubated with tritiated testosterone. We have had an opportunity to study two patients who were castrated. The tissues were obtained before and 90 days after castration, and the uptake of T is low in these patients with prostatic carcinoma. Conversion to DHT is present in the stroma alone, however. Affinity is low in prostatic carcinoma, but it does not change after castration in the two patients.

B. MOBBS: Dr. Mainwaring mentioned work that has been done in which it has not been possible to separate DHT binding by TeBG from receptor binding. It isn't possible to tell if the receptor is there or not. You mentioned our work where we found more binding present in carcinoma than in BPH. This is because many of these patients have been treated with estrogen which raises the TeBG level. So, we think this increased binding is at least partly due to increased TeBG. I believe Dr. Liao has an antibody assay that will enable us to eliminate binding to TeBG.

S. LIAO: Our methods are described in our recent papers (Endocrinol. Res. Commun. *1*, 271–281; J. Biol. Chem. *250*, 883–888, 1975). With antisteroid antibodies, the free steroids and nonspecific steroid binding can be eliminated. Using these techniques we have characterized the nuclear DHT-receptor of the human prostate as a 3S component. These methods are also useful in qualitative and quantitative characterization of other steroid-receptor complexes.

S. FANG: We have also tried to identify androgen-specific binding proteins in human prostate. We found that in many instances human prostate cytosol has to be pretreated with activated charcoal to remove the endogenous steroids before a 4S androgen-binding peak can be detected. It is mandatory, therefore, to remove the endogenous steroids before comparative measurements of steroid-binding proteins can be made on different samples. In regard to prostate 9S protein, it is important to take into account endogenous steroids and the kinetics involved in the formation of 9S complex. For instance, in cytosol that has been stripped of endogenous steroids by treating with activated charcoal and incubated with ^{3}H-dihydrotestosterone at 4°C, the formation of 9S complex keeps increasing even after three to four days. This means that depending on the length of time the cytosol is incubated with tritium labeled dihydrotestosterone, different pictures regarding the formation of receptor-steroid complexes can be observed. Another point of interest about the cytosol 9S protein is that once the complex is formed, it can be dialyzed against buffer for up to two days without any significant reduction in the amount of bound radioactivity. We suspect that the 9S protein may form some type of clathrate complex with steroid hormones. We should be careful, therefore, in considering the amount of receptor proteins because of the difference in conditions used in measuring the receptor protein. The above observation may also help to explain the failure of some groups to find specific binding proteins.

MAINWARING: We recognize the difficulties posed by these endogenous steroids. We attributed

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our failure to identify receptors reproducibily to this. The objective of this particular collaboration was not for comparative purposes but simply to do a qualitative study to determine if there is a specific binding protein present. We certainly never implied any comparison from one sample to another except that in half of the samples we could not find any specific binding.

O. HECHTER: Given the difficulties which have been discussed, it is not at all clear to me why anybody would want to measure androgen receptors in the prostate tissue one obtains from patients with BPH.

MAINWARING: This is a good question. I do not know where the future is going to lie. Dr. Mobbs says I am a pessimist; she may be right.

MOBBS: Well, the situation is analagous with that in breast cancer where many investigators are measuring estrogen receptors to predict whether a particular breast tumor is going to respond to hormonal therapy. This is the principal behind measuring androgen receptors in prostatic cancer anyway.

J. D. WILSON: The rationale for measuring androgen receptors in benign prostatic hypertrophy is to look for a species difference between the human prostate on the one hand and the majority of animals in which growth is distinctly limited. If androgen is administered to a bull, for example, the prostate grows, reaches a certain size, and stops growing; no matter how much additional androgen is administered, no additional growth can be induced. And that is true in a majority of species. In dog and man, there is asymetrical and continued androgen-mediated growth of the prostate. What we are looking for is a regulated function that will give us some idea of what limits growth. Once we understand about androgen receptors at a molecular biological level, it might be of theoretical importance whether there is a species difference of not.

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BENIGN PROSTATIC HYPERPLASIA: NATURAL HISTORY

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An understanding of the natural history of benign prostatic hyperplasia is obviously important in assessing prognosis and the need for surgery in the individual patient. It is of equal importance in evaluating the potential usefulness of newer chemotherapeutic agents. Unfortunately, this essential knowledge is not yet within our grasp.

Incidence

True nodular prostatic hyperplasia is seldom seen before age 40. In over 700 prostates studied by Moore (1), only one example was found in a younger man, aged 39, in whom the changes were microscopic in size. With advancing age, the incidence increases so that by age 80–90 approximately 75% of glands contain demonstrable hyperplastic spheroids.

The Canine Prostate

The dog is the only other species that regularly develops prostatic enlargement, but the process in the dog is different from that found in man in development, morphology and endocrine responses. In addition, although hyperplasia occurs primarily in older dogs—approximately 80% of those over 8–10 years exhibit it to some degree—it is not uncommon in younger animals.

The normal canine prostate ranges between 1–4 cm in diameter, depending on breed and age. After the 4th year, prostatic growth is enhanced in virtually all dogs, and the gland can become quite sizable; there is a case on record of a 10 lb. prostate in an animal weighing 25½ lbs. (2). It has been assumed that the hyperplastic process continues at a constant rate during the remainder of the life of the animal, but precise information on this point is lacking.

Histologically, the enlarged prostate in the dog differs from that in man in that it consists of diffuse, cystic hyperplasia with a heavy predominance of epithelial elements. This probably accounts for the relative absence of urinary obstruction, even in those dogs with huge prostates. This situation contrasts sharply with that in humans where the growth is nodular and primarily involves the periurethral tissues, compressing the urethra. An additional factor that may explain the absence of urinary difficulty in the dog is the comparative lack of fixation of the gland, enabling it to expand freely in all directions without necessarily encroaching on the urethral lumen.

Pathogenesis of Benign Hyperplasia

As described by Franks (3), the human prostate undergoes certain involutional changes early in adult life, which are focal at first but subsequently coalesce to form the typical fibroadenomatous lobes of benign prostatic hyperplasia. Stromal and epithelial elements in the inner or periurethral zone are primarily involved in this process, but associated changes also occur in the outer layer, i.e. the "true" prostate, or prostate proper. These latter changes consist chiefly of glandular atrophy (partly due to pressure from the expanding inner lobes), but secondary hyperplastic changes may occur here also. It is also in this outer layer that carcinoma originates.

The question as to whether the initial changes of benign hyperplasia take place in the epithelial or stromal elements of the inner layer of the prostate has never been satisfactorily answered. The prevailing view today favors the stroma as the site of origin of fibromuscular nodules arising from periductal and perivascular tissues, with secondary invasion, as it were, by nearby epithelial elements

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from the periurethral glands. In this way the well-known stromal-epithelial complexes are ultimately formed. In this connection Deming postulated the existence of an inherent factor in fibromuscular tissue capable of stimulating epithelial ingrowths into the primary nodule (4), a possibility that has never been resolved.

Subsequent Course of the Disease

The foregoing changes, which culminate in benign prostatic enlargment, are thought to begin in early adult life, but we do not know exactly when. Nor do we know precisely how rapidly they progress, although there is reason to suspect from histological studies that roughly 15–20 years are required from inception to the time when the disease becomes clinically manifest. This is about the same as in prostatic cancer, as estimated by Hirst and Bergman from an autopsy series in which they compared the incidence in routinely and serially-sectioned glands (5). These authors found the incidence of carcinoma on routine sections at age 75 to be the same as that on serial sections at age 55, hence suggested a 20-year lapse from the early stages of the disease to the time of clinical presentation. This reasoning by analogy is not without flaws, but it does provide some notion as to growth rate and may be applicable to benign hypertrophy as well.

As in the dog, information as to the natural history of benign hyperplasia in man is sadly lacking. Most urologists have the impression that the growth rate of benign prostatic hyperplasia is quite variable. This is based on the fact that in many patients the clinical picture may remain static for extended periods, i.e. months, even years, then suddenly become exacerbated, suggesting sudden resumption of prostatic growth. Or, conversely, it has been the feeling of some observers that patients may become asymptomatic for no obvious reason and may even exhibit apparent regression in prostatic size.

The questionable usefulness of such haphazard clinical data as the foregoing as valid measures of prostatic growth rate has prompted a number of studies in the past, all of which have been retrospective in nature. More recently, however, there have been two reports of prospective surveys of patients with prostatic symptoms, the first in England (6) and the other in this country (7).

The latter study deserves some comment. It was based on a systematic collection of data from 62 patients, 51 of whom were followed for over one year (average 210 weeks). Parameters measured included 1) symptomatology, 2) prostatic size estimated by palpation and radiographic findings, 3) urinary flow rates and 4) residual urine determinations. The results showed that, although all patients worsened in time, 24 had periods averaging better than one year where they actually improved without antibiotics or other medications. In addition, at any one time 53% of the total number of patients were either improving or apparently stationary. Objective regression in prostatic size was observed in some patients (number not specified). The authors concluded that although benign prostatic hyperplasia is admittedly a slowly progressive disease it seems to be characterized clinically in more than 50% of patients by periods of remission or at least temporary standstill. No prognostic indicators for individual patients emerged from the study.

Taken at its face value, this survey simply confirms the general impression urologists have formed over the years of the variability of the clinical course of prostatic hypertrophy among patients in general, and also in the individual patient. The authors, therefore, correctly point out the need for similar extended baseline evaluations prior to drug studies, not only in large number of patients but also in individual patients serving as their own controls. Although not specifically mentioned by the authors, the need for such a painstaking approach is underscored by the fact that the symptoms of prostatism are often not due to the hyperplastic process in and of itself but to those other commonly associated conditions, such as prostatitis, urethritis, prostatic calculi, infarcts, etc. All of these can cause symptoms, affect the size of the gland and influence the quality of voiding.

Postoperative Recurrence

Recurrence of hyperplasia following prostatectomy—or, more properly, fibromyoadenomectomy—is well-known. While in certain situations, recurrence undoubtedly develops from residual hyperplastic tissue inadvertently left behind by the surgeon, there is little doubt that true recurrence does develop within 3–10 years in some patients. It is not rare and sometimes requires repeated surgical intervention. Whether this secondary growth process is more rapid in the comparatively younger individual is unknown, but it is tempting to speculate that such may be the case in view of the higher growth potential in those in their 40's and 50's. This phenomenon certainly suggests that the growth

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process is not finite but has the capability of continuing throughout life

Conclusions:

In reviewing what is known about the natural history of BPH, we may conclude that:

1) Except for an educated guess that BPH requires about 15–20 years to progress from a small peri-urethral nodule of uncertain origin to a bona fide, clinical entity, virtually nothing is known about its actual course of development. Data available from clinical studies alone have shed little, if any, light on the subject.
2) A similar state of affairs exists with respect to prostatic hyperplasia in the dog, the only similar tumor occurring with regularity in the animal kingdom, at least in a readily available laboratory subject. Despite some similarities, spontaneous, canine prostatic hyperplasia differs in several important respects from its counterpart in man. Recent efforts to produce an acceptable model in dogs seem promising.
3) Finally, clinical impressions notwithstanding, I should like to express my personal view that prostatic hyperplasia in man is basically a progressive disease, albeit characterized by different growth rates in different individuals. There is no convincing evidence at present to support the notion that the hyperplastic process either ceases entirely or undergoes actual regression in the individual patient. Whether fluctuations in growth rate can occur, perhaps as the result of endogenous factors in the host, remains to be determined.

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DISCUSSION

D. S. COFFEY: Dr. Brendler, do you agree with what Dr. Farnsworth said in reviewing some one else's work that there is a relationship between prostatic CA and BPH? Is that your clinical impression too?

H. BRENDLER: I heard Dr. Fransworth say that, and it runs counter to my thinking.

COFFEY: Did you ever see a rapidly growing BPH?

BRENDLER: Well, that is hard to answer because all we have to go on are the parameters I mentioned before in describing the prospective study that was done fairly recently. These parameters are open to criticism as a valid measure of growth rate. However, I didn't mean to convey the impression that the growth rate is the same from patient to patient. It is just that I have never seen any solid evidence that the growth rate fluctuates in the same individual.

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EPIDEMIOLOGY OF BENIGN PROSTATIC HYPERTROPHY: REVIEW AND SPECULATIONS

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Very little hard information can be found that relates onset of BPH* to precursor variables, although there is a diffuse literature from which impressions can be derived. Mostly, it examines presumptive associations of BPH to one of several conditions: cirrhosis of the liver, diabetes mellitus, hypertension and other cardiovascular conditions, prostatitis, specified blood groups, and assorted endocrinologic hypotheses.

A limited body of information on worldwide mortality rates exists, and also a number of observations regarding such demographic variables as age, race, social class and marital status, sexual drive, physical constitution and other ascribed single-variable relationships have been made. There are no studies on the genetic contribution to risk of BPH. The relationship of prostatic hyperplasia to the subsequent development of prostatic cancer has been explored, as a possible linkage cannot be excluded from an epidemiologic review since it is a controversial issue. For this reason, I will present a speculative model that provides alternatives for the development of BPH as a separate entity, as an antecedent condition leading to neoplastic sequellae, or as a consequence of neoplasia.

The state of the art does not provide a single comprehensive epidemiologic case-control study specifically designed to generate worthwhile hypotheses that might contribute to a natural history of prostatic hyperplasia and serve to guide followup prospective studies and result in programs for prevention. Perhaps this follows from the feeling that since most older men have some degree of hyperplasia and since not too many will die from it large scale efforts and funding required for epidemiologic work would not be justified. Also, there may be some fatalism to this lack of research curiosity in the belief that the advanced ages at which men are diagnosed with BPH leave little room for salvage other than relief from symptomatology.

If morbidity and mortality data were better known such an outlook and neglect would be difficult to support. In some countries mortality rates for BPH are higher than those for prostatic cancer; and although it is true that prostatic hyperplasia occurs almost exclusively after the age of 40, there is a considerable incidence in the 50's and 60's, with most reports consistent in demonstrating highest frequencies in the 60's age group. Mean age by race for whites varies between 62 and 68 years and for blacks is about 5 years earlier, providing a considerable amount of opportunity for salvage. Advantage could accrue if identified variables occuring earlier in life were related to later onset of BPH.

Unless it can be shown that hyperplasia is a stage in the development of prostatic cancer as Armenian *et al* (1) recently claimed and Greenwald *et al* (2) denied, it would be difficult to speculate upon the origins of BPH from available information. If a relationship to cancer exists perhaps we can estimate safely the onset of the original cellular

*The terms hypertrophy and hyperplasia are used interchangeably in this paper to designate the clinically enlarged prostate and without regard to size or proliferation of cells. Both terms are found in the demographic and epidemiologic literature, often without reference to an exactly defined type of pathology. Choice of nomenclature appears also to depend upon geographic clinical usage by country.

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changes leading to BPH at some time during youth, since most solid cancers undergo a very long latent period, and prostatic cancer perhaps requires one of the longest since it arrives so late in life.

Clues are difficult to find, yet it is interesting that certain populations are at much lower risk of death from BPH than others. There is very little morbidity information which can be used for demographic comparisons. Excessive mortality seems concentrated in the more sophisticated European countries, where records and reporting are deemed reliable. Highest recorded mortality is in Iceland (3), closely followed by Germany, Austria, almost all of Scandinavia, and the Netherlands. Lowest mortality appears to be in those countries with Oriental populations, including Japan, Hong Kong, China (Taiwan), Philippines, Ceylon and Singapore. This freedom from BPH in Orientals is echoed also by extremely low death rates from prostatic cancer in the same populations.

International comparison mortality rates

The 45 countries represented in Table I are divided into 3 world areas by continent and adjoining territories, and individual populations are distributed by BPH mortality rates, providing comparison risk levels. All information is taken from WHO publications (3) with rates given upon a combined male and female population denominator. Reliability of these rates depends upon data furnished to WHO and vary in accuracy and dependability. Since rates shown in Table 1 apply to the total mixed-sex populations, they would be higher if applied only to a male population base. Furthermore, although the specific male segment at risk of BPH is age 40 and older, the assumption is that these rates are adjusted over the entire population of all ages.

As noted, virtually all populations at extreme risk of death from BPH are in or near Europe. For some of the reportedly low risk Oriental and Spanish-American populations true mortality might be masked by short life expectancies, but this is not applicable to Japan where life expectancy and the quality of medical care are parallel to those of Europe and the United States. Mortality for most of the American continent, including the United States, is quite moderate. Populations of Israel and Australia also are at moderate risk, as are those of Poland, England, Scotland, Ireland, the reported Slavic countries, and some other European countries. Some of these fairly low rates may result from inadequate reporting, incorrectly diagnosed causes of death, unavailability of medical care, and other biases, but this probably is less so for several of these countries, including the United States, Israel, England, Canada, and Scotland.

The American population for which a fairly high mortality from BPH is reported is in Chile. The other high-moderate death rates all are attributable to European countries. Average mortality from BPH for the world is at about 30 per 100,000 mixed-sex population, a very high rate exceeding those of many prevalent cancers and even higher if standardized only for males of ages 40 and higher. These death rates suggest that mortality from BPH may represent one of the most serious causes of death in some countries. The range is from 1 to 83 per 100,000, age-adjusted over a total mixed-sex population.

Association of BPH with demographic variables

The relationship of age to increased risk of prostatic hyperplasia has been reported in a number of studies (Table II). All agree that the highest frequency of BPH is between ages 60 and 70, peaking in blacks about 5 years earlier than in whites. Most males at around age 80 or older will be found with hyperplasia. Moore (4) and Lisoos (5) agree that earliest BPH cases are found at about 40 years of age, and Lisoos reported patients as young as 28 years and as old as 112 years in Bantus of South Africa. From these studies it is evident that benign prostatic hypertrophy seldom is found in young men, with frequency increasing in men over age 40.

Several studies have reported the extent of prostatic hyperplasia by geography and race, (Table III). Lisoos and also Dodge (6) reported BPH as commonplace in Bantus. For Wales, Ashley (7) reported an excess of deaths from BPH in areas with a high Welsh-speaking population, a smaller excess where fewer Welshmen were found, and a low rate where Welsh speaking was rare. He concluded that there is greater risk of BPH among Welshmen compared to English immigrants to Wales, based upon genetic differences between the two populations, expressed as degree of "Welshness" and identified by speaking the Welsh language, having Welsh surnames, and other Welsh characteristics. He admitted however that this significant excess of BPH cases might have resulted also from bias in the selection of patients for admission to an acute general hospital. Frequencies in blacks of New Orleans were reported by Derbes *et al* (8) and D'Aunoy *et al* (9) as parallel to or even greater than in whites.

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TABLE I—Ranked distribution of BPH mortality rates, 45 countries by continents. All rates per 100,000 mixed-sex population.[1]

Risk Level	Continent: America Country	Rate	Asia Country	Rate	Europe[2] Country	Rate
Low			Phillipines	1.2		
	Dom. Republic	5.1	Singapore	2.5		
	Mexico	8.7	Ceylon	3.2		
	El Savador	9.6	China (Taiwan)	4.0		
			Hong Kong	5.5		
			Japan	7.1		
Low Moderate					Poland	18.7
					Spain	19.6
	United States	14.4			Yugoslavia	22.2
	Trinidad	17.8			Australia	22.7
	Venezuela	18.6	Israel	24.1	Portugal	23.3
	Canada	20.5			N. Ireland	23.7
	Columbia	22.0			New Zealand	26.3
	Panama	23.3			Scotland	27.3
					Eng.—Wales	27.5
					Greece	29.8
High Moderate					Sweden	31.3
					Belgium	31.6
					Finland	31.8
					France	33.7
	Chile	38.3			Czechoslovakia	34.1
					Hungary	37.6
					Italy	42.4
					Bulgaria	42.7
					Ireland	42.9
					Malta	46.7
High					Netherlands	50.5
					Switzerland	52.1
					Denmark	53.1
					Norway	53.6
					Romania	55.9
					Austria	67.1
					Germany	75.1
					Iceland	82.5

[1] Average global mortality (unweighted mean of all rates): 30/100,000
[2] Includes Australia and New Zealand

TABLE II—Demographic variables related to benign prostatic hypertrophy: *age*.

Study	Factor	Population or Series	Results
Moore (*A*)	Age	St. Louis (series)	Age range: from 39 years with increasing incidence with age, but clinical incidence of urethral obstruction peaks at age 63 (Gover, 1923).
Moore (*B*)	Age	St. Louis (review)	75% of males 80 or older with BPH.
Derbes, *et al.*	Age	New Orleans (hospital data)	BPH frequency highest between ages 60–70; Mean age blacks: 62 yrs., whites: 67 years.
D'Aunoy, *et al.*	Age	(New Orleans (series)	Average age of admission for BPH, whites: 67 yrs., blacks: 62 yrs.
Lisoos	Age	Bantu (patient series)	Mean age BPH, 64 yrs.; range 28–112 yrs.
Richardson	Age	Scotland (statistical data)	Incidence of BPH increases with age; mean age at admission 72 years.

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Moore (4,10) in 2 studies reviewed rates in Orientals, blacks and whites, and concurred that BPH is rare in Orientals and common in whites and blacks. Secure studies in comparative frequencies of BPH still are few, and rates are not well established since many patients with hyperplasias are asymptomatic. There also seem to be differences of opinion regarding the establishment of hyperplasia as the only and actual cause of associated symptoms, such as urine retention.

Several additional demographic factors are examined in Table IV. In a study relating social class to risk of prostatic hyperplasia, Richardson (11) showed morbidity as higher in the upper classes of Scotland. However, on the usual 5 point socioeconomic scale used by British investigators in ranking socioeconomic status, an interesting result was that rates of BPH were lowest in Class 3, which by definition is a fairly well-to-do class, and the reliability of the relationship with social class appears uncertain. Marital status seems also unrelated. Moore's report (10) is quite old, but his review has not been challenged. He and also Van Der Ries (12) reported BPH as common in Catholic priests as in the general population, excluding celibacy as a risk influence. If true, celibacy does not protect men against prostatic hyperplasia as it does women against cervix cancer. If BPH is a condition that leads to prostatic cancer, as has been claimed (1), and if there is an agent that initiates the development of hyperplasia, it might seem not to be sexually transmitted. Certainly, extended studies on this issue are indicated.

Sexual drive was reviewed by Moore (10) and by Van Der Ries (12) with concurrence that it seems not to be related to increased risk. It is difficult to resolve the measurement of "sexual drive," as also it is to understand the rationale for selection of this variable from among many other sexual variables which are more appropriately investigable. Progress has been made in the study of sexuality since these early studies, but application to risk of BPH has not yet taken place. Blatt, cited by Moore, (13) endowed the BPH patient with feminine charac-

TABLE III—Demographic variables related to benign prostatic hypertrophy: *geography, race.*

Study	*Factor*	*Population or Series*	*Results*
Lisoos	Geography, Race	Bantu	BPH not uncommon in Bantu.
Dodge	Race	Bantu	BPH common in Bantu.
Ashley	Geography	Wales (Hospital data)	Greater susceptibility of Welshmen to BPH ("due to inherent factors").
Derbes, *et al.*	Race	New Orleans	Frequency of BPH at least as great or greater in blacks than in whites.
D'Aunoy, *et al.*	Race	New Orleans	Incidence of BPH greater in blacks: 55% vs 45%.
Moore (*B*)	Race	Review	Incidence: Japanese "rare"; Chinese low; whites high; blacks as high or higher.
Moore (*A*)	Race	Review	Rare in Chinese; common in whites and blacks.

TABLE IV—Demographic and risk variables related to BPH: *social class, marriage, sexuality, physical features.*

Study	Factor	Population or Series	Results
Richardson	Social Class	Scotland	Death rates from BPH higher in upper classes. Morbidity higher in "well-to-do". Lowest rate in Class III.
Moore (*A*)	Marital Status	Review	BPH is "slightly less common" among single men. As common in Catholic priests as in gen. pop.
Moore (*B*)	Sexual drive	Review	No "variation" in sexual drive.
Van Der Reis	Sexual drive, celibacy	Review	No difference; incidence in celibates equal to that of noncelibates.
Moore (*B*)	Constitution, physical features	Review	Cites Blatt: comparison of feminine features and BPH patient, some similarity. (Unconvincing).

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teristics, but this has not been reported elsewhere.

Risk relationships

The interaction of risk-related attributes with prostatic hyperplasia may be approached from two reference positions: 1) the claimed effect of several endogenous variables upon risk of BPH, and 2) the possible development of prostatic hypertrophy into prostatic cancer.

Investigations of the effect of hormones upon etiology and risk of BPH have been concerned with androgen/estrogen balance, the influence of androgenic action upon prostatic growth, hyperestrogenism, relative pituitary, adrenal and gonadal size and weight, and other variables. In any case, the relationship of hormones to BPH is a complicated subject, and since much of the material presented in this workshop is endocrinologic no further mention of this area will be made in this review, although there is epidemiologic relevance in the sense that genetic variables may operate upon hormonal function and status in relation to risk.

Van Der Ries (12) investigated the relationship of coronary heart disease to risk, but he regarded the discovered correlation as "coincidental," (Table V). Since both BPH and coronary heart disease are highly prevalent in older men such a spurious correlation might be expected, as also would be true of other prevalent conditions in older men. From autopsy and surgical series, Odunijo and Elebute (14) concluded that the firm nodular type of BPH may be related to earlier prostatitis. This is a subject that often is discussed in relation to hyperplasia and neoplasia of the prostate, yet the problem of collecting reliable data would seem to be insurmountable except in carefully controlled longterm longitudinal prospective studies, since inflammatory prostatic conditions so often are found in younger men and easily could remain undisclosed or unresolved 30 or 40 years later. Prospective studies of this type have not been attempted, perhaps for the better. The probability of conducting such studies successfully over long periods of time is slim, and the hypothesis itself remains doubtful. Bourke and Griffin (15) demonstrated high frequencies of hypertension in BPH patients, possibly another fortuitous correlation based upon high prevalences with advancing age; and the same authors found no relationship of BPH with any preferential ABO or Rh blood groups (15). From hospital records covering autopsy series, Robson (17) found only expected frequencies of BPH in studied cirrhotics.

Two authors have reported on the possible relationship of diabetes mellitus to BPH. Roberts (18, 19) speculated that diabetogenic hyperinsulinism stimulates androgenic action with a consequent effect upon prostatic growth. The influence of growth, adrenocortical, pituitary, and interstitial cell stimulating hormones tend to "exaggerate" this effect; and the high zinc, fructose and enzyme content of prostatic and seminal fluids favor persistence of the local hyperinsulinized state which provides the effect on androgen secretion and main-

TABLE V—Factors associated with risk of BPH: ***cirrhosis of liver, Diabetes mellitus, coronary heart disease, prostatitis, hypertension, blood groups.***

Study	Factor	Population or Series	Results
Robson	Cirrhosis	Maryland (Hospital records for autopsy series)	Cirrhotics not found to have a greater than expected incidence of BPH *or* Ca.
Roberts	Diabetes Mellitus	Review, hypothesis	Relationship to pathogenesis of BPH.
Bourke	Diabetes Mellitus	England (patient series)	High incidence in BPH patients (>50%). Suggests increased estrogen secretion with advancing age.
Bourke	Diabetes Mellitus	England (Population data)	Incidence of diabetes "significantly raised" in BPH series, compared with general population.
Van Der Reis	Coronary Heart Disease	South Africa	Correlation between BPH and CHD is "coincidental".
Odunijo and Elebute	Prostatitis	Africa (autopsy and surgical series)	Type II (hard, nodular) BPH may be related to chronic prostatitis.
Bourke and Griffin	Hypertension	London (patient series)	Greater incidence of hypertension in BPH patients.
Bourke and Griffin	Blood groups	London	No association with any group, ABO or Rh.
Bourke and Griffin	Blood groups	London	No association with any ABO or Rh group.

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tains excessive growth. No evidence accompanies this highly structured theory, which is presented here in extremely simplified form; but Bourke and Griffin (20) collected data from a series of patients and also from populations in England which demonstrated a significantly higher incidence of diabetes in patients with prostatic hyperplasia. These authors proposed the influence of increased estrogen formation that occurs with advancing age, but the possibility exists again that this relationship is a statistical one based upon high numbers of older men with both BPH and diabetes mellitus.

The relationship between benign prostatic hypertrophy and prostatic cancer long has been a controversial subject, and the possibility that cells in an already pathologic and hyperplastic matrix can develop into a carcinoma cannot be overlooked. Reports of the coexistence of BPH and prostatic cancer are scattered throughout the literature, (Table VI). That prostatic carcinoma is found in many BPH patients is not surprising since most older men have some degree of hyperplasia and since prostatic cancer also is quite common. The studies of Labess (21), Hunt (22), and Myers (23) demonstrated such an expected finding, although percentages differed. Dodge (6) found BPH in half of prostatic cancer cases in Uganda, but he admitted that BPH is common among these African people. These reports all are quite old, dating back as far as 1929.

It would be safe to say that until quite recently most, if not all, urologists regarded hyperplasia and neoplasia of the prostate as two separate and unassociated conditions. However, two recent reports have raised the issue of relatedness, each completely opposed to the other. Armenian *et al* (1) described a "prospective" study in which 296 BPH cases and 299 age-matched controls were traced until death or until the end of 1972, all cases having been diagnosed between 1945 and 1965. The death rate from prostatic cancer was almost 4 times higher in the BPH group than in the control group (Table VI). In a separate study, with data obtained from hospital records and reported in the same paper, these authors compared 290 prostatic cancer cases against 290 age-matched controls. From this they derived a relative risk of 5 for diagnosis of prostatic cancer in patients who earlier had been treated for BPH. At about the same time Greenwald *et al* (2) at the Lahey Clinic in Boston reported a study in which 838 patients with BPH and 802 age-matched controls were followed for 11 years. Similar numbers of patients and controls developed prostatic cancer (Table VI) They also reported no significant differences for a number of other variables including marital status, frequency of bladder cancer, diabetes mellitus, use of drugs, family histories, smoking and drinking. This study, which truly was prospective in design, runs contrary to the report of Armenian *et al*, and from these results it would appear that BPH and prostatic cancer have separate etiologies.

The difficulty of designing BPH case-control studies is apparent from both reports. As an absolute necessity, a convincing level of evidence is required for each prostate to demonstrate that initiation of cancer already has not taken place before onset of BPH. Studied subjects of the Greenwald project had undergone subtotal prostatectomies, mostly by the transurethral route. A criterion for inclusion in the study was the requirement that pathology reports on resected tissues from transurethral and suprapubic prostatectomies be negative for cancer. Solid evidence that each prostate was free of neoplasia was limited since adequate biopsy procedures or total removal of these prostates would have been required for verification. The Greenwald study seems reasonably well designed and conducted in view of difficulties associated with reliability based upon pathologic determinations as

TABLE VI—Association of prostatic cancer with benign prostatic hypertrophy.

Study	Factor	Population or Series	Results
Armenian, *et al.*	Prostatic Ca.	Buffalo, N.Y. (case-control)	Death rate from pros. ca. 3.7 x higher in BPH group. Relative risk 5.1 in patients seen earlier with BPH.
Labess	Prostatic Ca.	Philadelphia (consecutive series)	9.2% of BPH patients: coexisting prostatic ca.
Greenwald, *et al.*	Prostatic Ca.	Boston	No relation: 24 BPH patients; 26 controls developed ca. in 11 yrs.*
Hunt	Prostatic Ca.	Minnesota (case histories)	Prostatic ca. found post-prostatectomy for BPH.
Myers	Prostatic Ca.	Pueblo, Colorado (series)	Ca. prostate in 29.4% of BPH patients.
Dodge	Prostatic Ca.	Uganda Africans	BPH found in 26/50 ca. cases, but BPH common in Uganda.

*No significant differences for marital status, bladder ca. freq., diabetes, drug-use, family history, smoking, drinking.

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opposed to clinical findings, especially since studies of this type involving indeterminate and uncontrollable biases and variables are more easily criticized than organized and carried out. The Armenian report also suffers from a number of weakneses. Although some BPH cases were biopsied, absence of cancer was not verified in others at the time of admission for hyperplasia. It is entirely possible that latent or even low grade invasive cancers might not be found with hyperplasias which were detected only clinically. Further, patients with BPH were reviewed many times, controls only once, introducing the possibility of selection bias. Further selection might have taken place in the control group since 36 patients with enlarged prostates were excluded, as Franks (24) already has pointed out.

However, most important is that these authors suggested that cancer develops from hyperplastic tissue, which they regard as "biologically plausible," but this is hardly plausible when compared with models of carcinogenesis in animals and also in humans (25). It is not likely that a condition found late in life such as prostatic hypertrophy would be antecedent to a solid carcinoma, which is known to have a long latent period. Latent cancers are found in young men and also in men at low risk of clinical cancer, as in Orientals; whereas prostatic hyperplasias are seldom found in men under age 40. A biologically plausible explanation is that initiation took place before hyperplasia; if there is an association, it must be that hyperplasia is one of the possible sequellae of the developing carcinoma. In view of these difficulties and the opposing results of the Greenwald study, acceptance of the Armenian results will depend upon repeated studies with similar trends. Until more evidence accumulates, the belief that prostatic hyperplasia increases risk of cancer must be held in abeyance in favor of the hypothesis that both conditions are independent or that cancer increases risk of hyperplasia, perhaps with a commonality of origin. Resolution of this issue awaits a convincing natural history of prostatic hyperplasia based upon combined epidemiologic and pathologic studies.

Worldwide risk factor/mortality rate comparisons

A favored epidemiologic pursuit has been to correlate morbidity or mortality rates of 2 diseases for a number of countries to hypothesize a presumptive relationship of one to the other. The technique is to plot rates in pairs for each country as scattergrams and then to test for association with correlation coefficients and regression trends. Since accurate morbidity rates are not often available, death rates usually are used. Wynder, *et al* (26) provided such a series of mortality comparisons, and a number of these resulted in positive correlations.

There are 4 possible reasons for such a positive result: 1) both conditions may share a common pathogenesis, 2) both conditions are competing causes of death at a given time in life, as in old age, 3) one condition is a pathologic precursor of the other; that is, one disease develops into the other, and 4) the relationship is fortuitous and is based upon chance, poor or untreated data, selection bias arising from choice of countries to be compared, or the use of mortality rates to represent the true distribution of each condition in the studied populations.

Wynder *et al* regarded the use of general mortality rates instead of age-specific rates as a disadvantage. Since this is especially true for conditions such as BPH which are limited to certain age groups, the utilization of mortality rates for these comparisons is open to question.

An example of such a death-rate bias is the comparison of mortality from prostatic hyperplasia and prostatic cancer in different countries. There is a substantial amount of mortality from both causes, and morbidity for each is much in excess of mortality. Both are associated with advancing age, and a seeming relationship may result from the effect of competing causes of death on the same organ. For studies of this type only morbidity rates are convincing, but mortality studies, however weak, may provide clues.

With these observations in mind, several such comparisons are made in Figure 1 to probe for a possible effect from claimed risk variables upon mortality from prostatic hyperplasia. The references from which these rates were taken do not indicate that they are specific for the age-range to which each entity is related or that these rates are standardized upon an accurate and contemporary population base for each country. Also, reporting seldom reflects the true causes of death and, as a consequence, when such comparisons are made trends can be manufactured simply by including or excluding countries. This takes place mechanically as a result of the lack of data from certain parts of the world.

From these scattergrams, each shown with its regression of one variable upon BPH mortality, it appears that death rates from benign prostatic hyperplasia are strongly correlated with death

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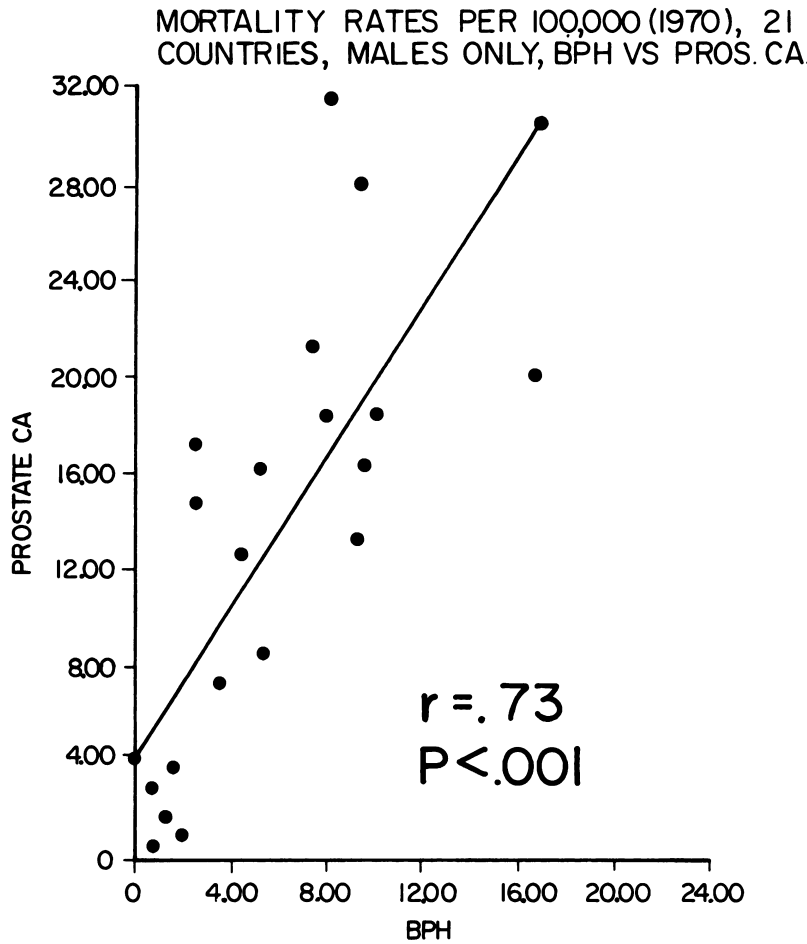

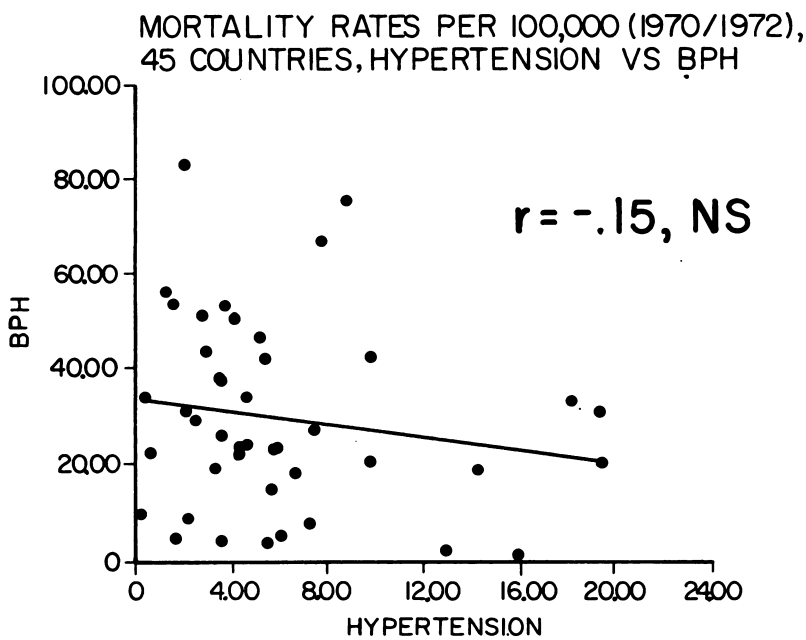

FIGURE 1. Regression trends and correlation coefficients to test

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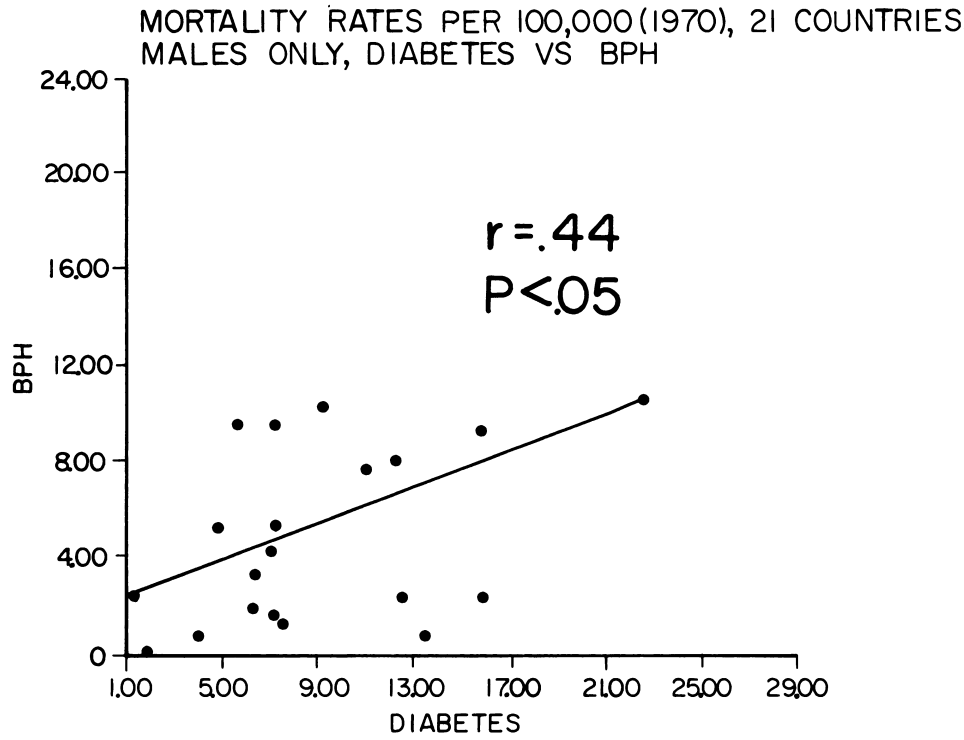

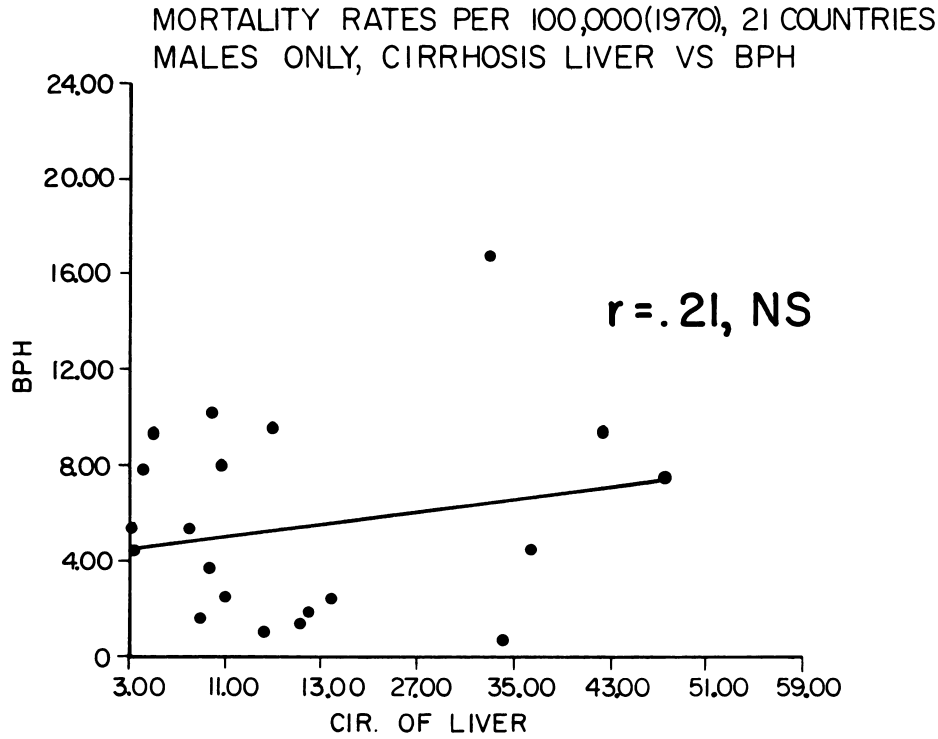

the association of mortality from BPH with mortality from selected causes.

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rates from prostatic cancer in the 21 represented countries where rates are limited only to the male population. A positive but weaker relationship is shown between diabetes mellitus and BPH, and a possible but weak association exists between cirrhosis of the liver and BPH, both comparisons being based upon the male populations of 21 countries. For the effect of hypertension and the BPH upon each other, mortality rates were available for 45 countries with the total male and female population as a base (3); no obvious degree of correlation was evident. Inferences derived from these comparisons may lead one to hypothesize or to reject etiologic commonalities; but to assume a direct sequential relationship of BPH with prostatic cancer, diabetes or cirrhosis of the liver is not justified even where the regression coefficients are positive and considerable.

Speculative rationale of events and relationships: BPH and prostatic cancer.

Figure 2 is an attempt to deal with the ascribed, controversial relationship of benign prostatic hyperplasia with prostatic cancer in terms of theories of carcinogenesis. Based upon an assumption of prepathogensis "normal" prostatic tissue, there are 5 tracks over which pathology can develop: 1) as a separate and independent chain of events leading to hyperplasia, detection, treatment, and survival or death, shown at the top of the diagram; 2) as a development of stationary neoplastic tissue either directly from the normal or as a progression from prostatic hyperplasia and ending as a latent carcinoma (A) which will develop no further, shown as the middle track in the diagram; 3) as a sequence from normal tissue directly to a developing neoplasia or as a sequel to hyperplastic tissue, resulting in the first stage of a carcinoma but this time with potential for progression into invasion; 4) as an effect where BPH develops as a consequence of an established cancer, and 5) as 2 conditions, BPH and cancer, both of which share a commonality of origin. Two separate A stages are postulated, one of which remains stationary and another which is progressive. Both are found as latent cancers although with a difference in capacity to proliferate further and to develop from a low grade lesion into a high grade malignancy. The model from which the progression of the invasive lesion through all its stages is taken was derived from Whitmore (27), with appropriate changes, to show sequellae of the third track. From this, it is assumed that stage A can progress through all subsequent stages to detection, survival or death and that stages B and C can progress or remain stationary.

From models of carcinogenesis, it is assumed that an event arises early in life that somehow transforms susceptible tissue and initiates the carcinoma, indicated as Risk Event 1. A theory that hyperplasia can lead to neoplasia also requires such a risk event with early cellular changes which develop into BPH, then progress into cancer. The question is: are these the same or separate risk events?

Models for carcinogenesis require further a second much later risk event to transform the *in situ* cancer into an aggressive lesion, usually labelled as *cocarcinogenesis* or *promotion*, shown as Risk Event 2. This obviously does not take place in the hypertrophied prostate unless the hyperplastic matrix becomes part of the continuum leading to cancer, shown by the diagonal double-arrowed line from BPH to the A stage of early cancer, to indicate the possibility also that a carcinoma has been initiated before onset of BPH. A number of questions arise: is there a separate risk situation transforming hyperplastic cells into neoplasia? Is this the same as Risk Event 2 (in which case there is a true multistage continuum model)? Are BPH and prostatic cancer separate and independent conditions? Are there *3* separate conditions, BPH, a latent stationary stage A cancer, and the fully developed carcinoma arising from a different A lesion, shown by the dotted line from latent A to B, and progressing to malignancy? Since solid carcinomas require long latencies, is the BPH superimposed upon an already existing cancer?

For development into neoplasia, a first event that initiates the proliferative and cellular changes characteristic of BPH might occur very early in life, perhaps during adolescence or later. If this is true, such an event might be sexually based and this is where inquiries should be directed, although these changes well could take place in the 20's or even as late as the 40's. For such late changes a sexual event is even more suspect. This could relate to changes in sexual behavior which begin for many men in cultures with highest mortality from both BPH and prostatic cancer during middle age and accumulate thereafter with advancing age. In these countries cessation of sexuality in males begins at about the time of life when frequencies of BPH begin to accumulate, perhaps with changes which can operate upon early prostatic lesions as a cocarcinogenic event. Whether this is applicable to prostatic hypertrophy is hard to say. In such a case the initiation stage of carcinogenesis might be by-

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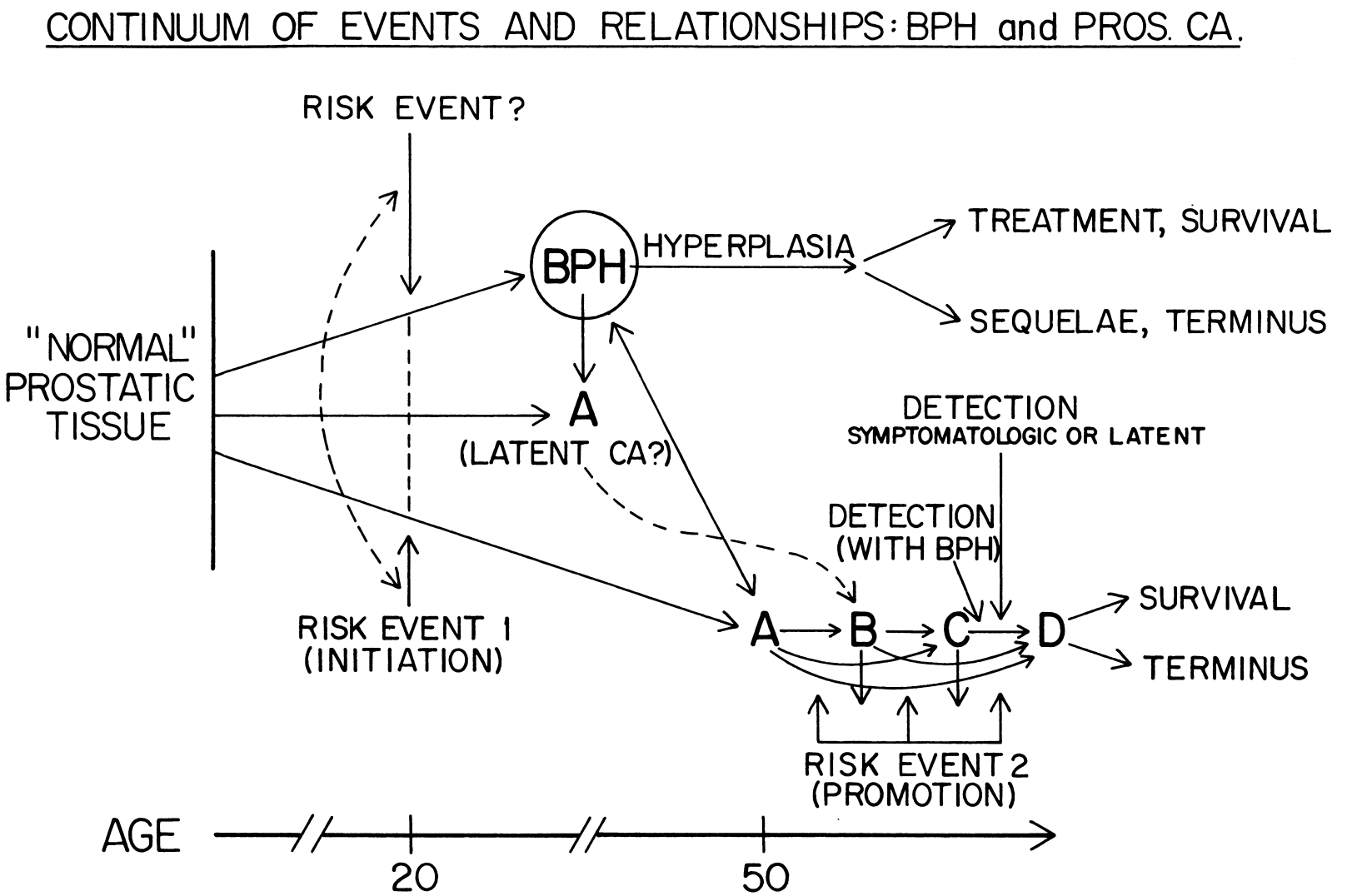

FIGURE 2. Hypothetical model for the development of BPH as a separate condition, as a precursor to onset of prostatic cancer, or as a sequel to an already established carcinoma. See text.

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passed, but the later promotional event might be operational. Perhaps also the chain of events is controlled by a genetic timetable with BPH related to endogenous factors; whereas carcinogenesis is initiated by exogenous oncogenic or carcinogenic variables related to sexual patterns. For this, both conditions must be separate entities.

Concluding observations.

Very little is known about events contributing to the risk of benign prostatic hypertrophy and also about what happens to prostatic hyperplasia after it develops. That hyperplasia in the prostate is a condition that increases risk of prostatic cancer is not established securely; from evidence to date BPH and prostatic cancer appear to emerge as separate pathologic entities. Much more investigative work will be required to clarify questions presented here, particularly since there seems to be a great deal of mortality from benign prostatic hypertrophy in the world. Probably a series of well designed case-control epidemiologic studies would be useful, although rigorous criteria for admission of cases and controls into such studies might be impossible to follow. An acceptable compromise might be to qualify controls as free from BPH by clinical examination and without requirement for microscopic pathologic assessment. Such a procedure still would not satisfy the need to determine preexisting carcinomas in studied BPH subjects.

Summary

Mortality rates for benign prostatic hypertrophy are high in Iceland and in most European populations, low-average in U.S. men, and lowest in countries with Oriental populations. Average global mortality is about 30 per 100,000 mixed-sex population. Although morbidity rates are largely insecure or unknown, it is estimated that 75 percent of white and black males past age 70 will be found to have BPH on examination. Mean age of detection by race is about 65 for whites and about 5 years earlier for blacks. The disease is seldom diagnosed in males under age 40.

From limited available reports, risk of BPH seems unrelated to social class, marital status, celibacy, "sexual drive," or any specific ABO or Rh blood group. Studies appear to demonstrate that excesses of men with coronary heart disease, hypertension, diabetes mellitus and cirrhosis of the liver may be found with BPH, but some or all of these ascribed associations may fortuitously result from increasing frequencies of all these conditions in older men. Death rate correlations reveal a significant association only for diabetes with BPH, perhaps also based upon competing causes of death for both diseases in older males.

From recent conflicting reports, the claimed association of BPH as a precursor condition developing into prostatic cancer must be held in abeyance pending more evidence, since a likelihood exists that BPH develops after initiation of the cancer or that both entities are separate conditions. Global death rates for both diseases are correlated, significantly, but this might be expected for 2 prevalent old-age related diseases, even if they are etiologically independent. Based upon models of carcinogenesis, a speculative rationale for possible events and consequences arising from such an association is presented.

Little is known about antecedent events contributing to the pathogenesis and increased risk of benign prostatic hypertrophy. The body of information is limited, diffuse and insecure. Although very difficult to design and carry out, epidemiologic studies are necessary in view of high mortality rates.

I am grateful to G. Vercelotti for help in searching the literature; and to V. K. Moses for assistance in assembling data from world references.

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DISCUSSION

O. A. HAUGEN: I would like to describe evidence that demonstrates the high frequency of BPH in an autopsy population. The autopsy rates are high in all the Scandinavian countries, being approximately 85% at Ullevål Hospital in Oslo, Norway. I have been unable to find a single case of BPH below the age of 40. This supports the conclusion made by Moore that BPH does not develop before the age of 40. After the age of 40, however, there is a very strong age-related increase of BPH, reaching 95% in the 8th decade. To find a histologically normal prostate after the age of 70 is rare. This has some bearing on clinical studies on the prostate in which individuals with a "normal prostate" are used as controls. It is not possible to find truly normal prostates in older men since prostates of normal size or even with subnormal weights often show microscopic foci of benign hyperplasia. By digital examination alone, it is impossible to exclude the presence of benign hyperplasia. We have also studied prostates of patients with cirrhosis of the liver and we have been unable to find any altered frequency of benign hyperplasia or carcinoma in this condition compared with age-matched controls.

M. TANNENBAUM: Another variable that has been ignored, though well documented in the literature, is the compulsiveness of the pathologist. If one cuts a single section form a prostatic surgical specimen you will find a 3–4% incidence of carcinoma of the prostate. If multiple sections are taken for histologic examination, and this has been documented in autopsy specimens by Angrist, then the percentage of carcinoma in these surgically removed prostates as well as autopsy specimens will be 30–40%. When evaluating data such as this, one has to be sure that all the prostates are blocked out and that enough sections are taken for a true incidence of carcinoma of the prostate to be documented.

I. D. ROTKIN: That's the reason why in so many of these studies, if we undertook epidemiologic surveys, we would be dealing with uncertainties that render information insecure.

M. TANNENBAUM: The other difficulty is that there are times where a gradual transition from so-called "atypical hyperplasia" of BPH to that of carcinoma occurs imperceptibly. These areas can be misjudged when there is cautery artifact.

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BENIGN PROSTATIC HYPERPLASIA: EVALUATION AND ASSESSMENT OF BLADDER NECK OBSTRUCTION

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The clinical problem of bladder neck obstruction secondary to benign prostatic hyperplasia results primarily from an imbalance between intravesical voiding pressure and outflow occlusion at the level of the prostatic urethra. The art of evaluating the degree of symptomatology evidenced by the patient, primarily a subjective evaluation, is termed clinical medicine; the science of objectively determining the degree of prostatic outflow obstruction and its physiologic consequences is termed urodynamics. Evaluation of the patient with prostatic outflow obstruction should properly embrace both the art and science of medicine.

The standard methods for evaluating patients with clinically significant prostatic outflow obstruction include (a) the clinical history, (b) observation of the voiding stream, (c) physical examination including cystourethroscopy and (d) radiography.

Clinical history

The patient with bladder neck obstruction secondary to prostatic enlargement will present complaining of a decrease in the size and force of his urinary stream, of having to strain to initiate voiding, of daytime urinary frequency, of an increase in the number of episodes of nocturnal voiding, of difficulty in halting the urinary stream, of postvoiding dribbling, of a feeling of incomplete emptying and, occasionally, of persistent dribbling and periods of total urinary retention (1,2,3). Some or all of the symptom complex may be present. The number of complaints and the severity of the symptoms will vary from patient to patient and will depend on his own degree of symptom tolerance. There are those patients whose degree of anatomic prostatic outflow obstruction is significant, yet whose verbalized symptomatology is minimal as they tolerate the discomfort with little difficulty. Conversely, there are those patients who are troubled greatly by only minimal degrees of anatomic obstruction as their threshold of discomfit is low. How does the physician clinically evaluate the presenting symptomatology?

The Physical Examination

The examining physician should observe the patient during voiding to confirm straining at urination with decrease in size and force of the urinary stream. Examination of the lower abdomen may demonstrate a distended bladder while rectal examination will demonstrate an enlarged prostate. Finally, cystourethroscopy should demonstrate that the prostatic urethra is lengthened and occluded by a mass of enlarged prostatic tissue. The bladder may show the effect of prolonged outflow obstruction with hypertrophy of the bladder muscle fibers (trabeculation) and multiple small outpouchings of bladder mucosa between these fibers (saccule formation). Intravenous pyelography may show angulation of the distal ureters by the enlarged gland and a failure to empty the bladder of contrast material after voiding (3). No numerical correlation can be made between the amount of contrast material remaining in the bladder after voiding and the actual amount of urine not voided. Symptom correlates do not exist between the radiographs and the patient.

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Although the historical setting should identify accurately the patient's response to his anatomic obstruction, the subjective parameters outlined above provide little more than a mental image of the degree of bladder neck outflow obstruction. Therefore, much complex instrumentation has been developed to provide objective numerical indices to establish reproducible and comparable data on the severity of bladder neck obstruction from prostatic enlargement. Multiple mechanisms for assessing intravesical tone, the force and strength of the urinary stream, the rate of the urinary flow, the amount of urine remaining within the bladder after voiding and the resistance to flow across the prostatic urethra have been reported.

Post-voiding Residual

An early method for measuring the efficiency of bladder emptying was the measuring of the urine remaining within the bladder after voiding was completed. This, the post-voiding residual (normally less than 30cc) was obtained by passage of a transurethral catheter after voiding. Recently, radionucleotide scanning of the emptied bladder after administration of a rapidly excreted radiolabel has provided a non-invasive method for assessing the post-voiding residual (4). Irrespective of the methodology employed, the post-void residual is elevated consistently only in those patients with a considerable degree of intravesical pressure (outflow obstruction imbalance) and does not correlate closely in the average patient with cystoscopically-proven anatomic prostatic obstruction (5). The post-voiding residual volume cannot be used to provide reproducible data on the degree of bladder neck obstruction.

Uroflowmeters

The first clinical uroflowmeters were developed approximately 25 years ago in an attempt to find accurate and reproducible methods for assessing peak and mean urine flow rates (6–15). As early as 1948, Drake recorded urinary flow by monitoring the weight of the urine (6). Since that date, other investigators have employed the same principle with various refinements. In 1963, a new technique was described by Cardus and his associates based on the variations of a constant magnetic flow due to the electrolytes contained in urine flowing through a funnel (7,14). Urine flow has also been recorded by voiding on a disc which rotates at constant speed. The disc is slowed by the urine flow, and the power needed to keep the disc at constant speed is proportional to the flow. Air displacement principles were first used by Palm and Neilson in 1967 (15) and in 1969 by Holme (13), who measured the maximum flow by recording the volume of air displaced by a proportional volume of urine. Sussett and associates recently developed a uroflowmeter based on air displacement (1). The air cools a hot wire, and the amount of power needed to keep the wire at 180°F is proportional to the urine flow.

In the normal male, the mean peak urinary flow rate is 30cc/second, varying from a low of 20cc/second to a high of 38cc/second (5,10,11 and 16–20). (Fig. 1) The peak urinary flow rate varies with volume, being least at low bladder volumes; however, the curve levels off above 200cc and has even been felt to be valid if as little as 100cc is voided (11,20). The mean urinary flow is more responsive to volume voided with the average mean urinary flow being about 22cc/second (5,11,19,20). (Fig. 2) The uroflowmeter curve in the normal male subject is sharply peaked in contrast to the broad flat curve seen with bladder neck obstruction (20 21). Sussett et al recently evaluated the various types of uroflowmeters and determined that uroflowmetry is an adequate method for assessing prostatic outflow obstruction (11). The value of the peak and mean urinary flow rate in evaluating prostatic outflow obstruction also was demonstrated by Paulson and Kane, who showed an improvement in both peak and mean urine flow rates in patients receiving progestational agents for pharmaceutical control of benign prostatic hyperplasia with the degree

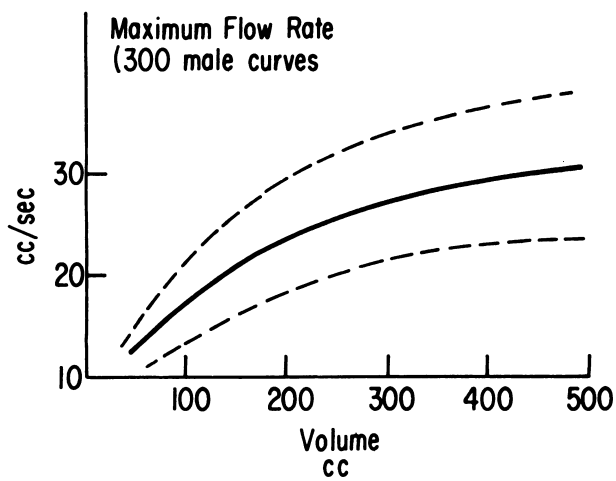

FIGURE 1. Peak Urinary Flow as a Function of Volume Voided. From Sussett et al. J. Urol. 109: 874–878, 1973.

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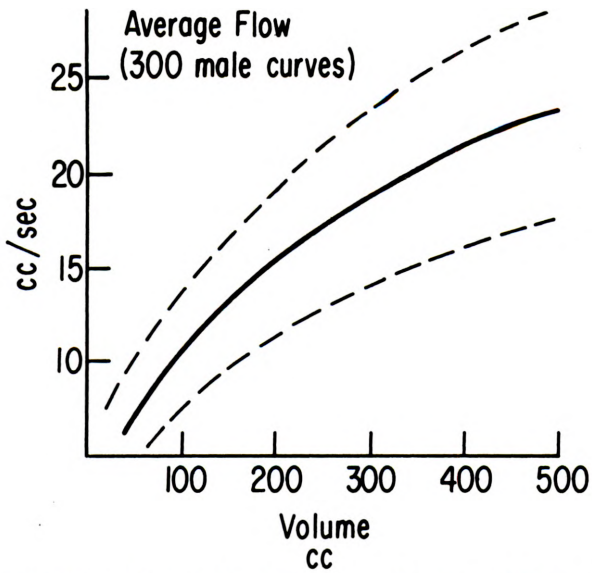

FIGURE 2. Mean Urinary Flow as a Function of Volume Voided. From Sussett et al. J. Urol. 109: 874–878, 1973.

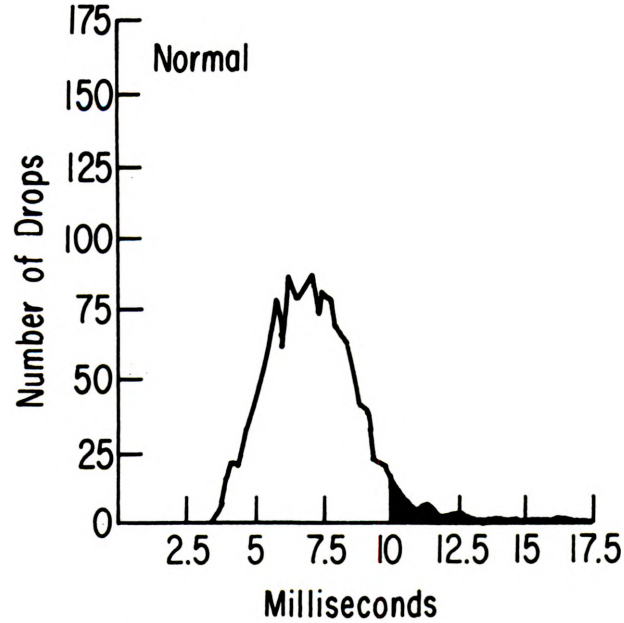

FIGURE 3a. Drop Temporal Interval Distribution in Normal Man.

of improvement recorded related to the amount of drug received (5). Zinner and associates, having demonstrated that the urinary stream breaks into multiple droplets shortly after emerging from the urethra, have developed a complex electronic system for determining the drop temporal interval distribution within the urinary stream (22,23). Herein, the data is recorded in the form of electronic pulses, each pulse resulting from the passage of a drop of urine through an optical beam. The pulses are subsequently stored and displayed on an oscilloscope at the termination of the recording. It has been demonstrated that the patients with BPH demonstrate a broad flat histogram with the curve similar to that seen with the uroflowmeter. (Figs. 3a, 3b) There are significant technical problems in the development of this methodology, and with the present analyzer used each electrical signal representing a drop of urine is evaluated by the edge of the drop triggering the signal. A more sophisticated means of data processing would be to trigger on the drop centroid.

Prostatic Urethral Resistance

The methodology discussed previously serves to measure the urinary flow rate and degree of bladder emptying as a single event and does not relate dynamically intravesical pressure and prostatic urethral resistance. Intravesical pressure is developed both by detrusor contraction and by intra-abdominal pressure increases produced by contraction of the abdominal musculature. An increase in rectal pressures, from the resting values (mean 22.6mm Hg) when micturition begins, has been noted in over 50% of patients with clinically significant benign prostatic hyperplasia and is good

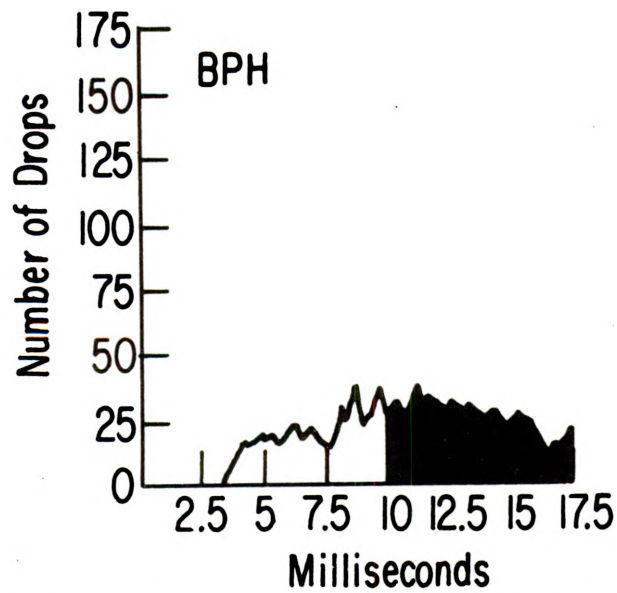

FIGURE 3b. Drop Temporal Interval Distribution in a Patient with BPH. From Zenner et al. J. Urol. 106: 115–121, 1971.

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evidence that prostatic patients strain to initiate micturition (20,21). Once micturition has been established, straining is not so significant. Intravesicle pressure can be measured by placing a small pressure catheter into the bladder either suprapubically or transurethrally. von Garretts demonstrated that urethral catheters with less than 4.9mm outer diameter did not influence bladder pressures during voiding in men and that flow rates were altered only by larger catheters (24). Gleason and Lattimer showed that intraurethral tubes of 5F (1.5mm outer diameter) did not cause flow rates to be significantly different from those obtained during free voiding (25). By placing a 5F catheter across the urethra into the bladder and a second 5F catheter in the bulbous urethra, simultaneous pressure within the bladder and urethra can be determined while urinary flow rates can be recorded (20,26). (Fig. 4) Lenaghan, using this methodology, has determined that the pressure gradients across the prostatic urethra, the "urethral prostatic resistance", is an extremely accurate index of the degree of prostatic outflow obstruction (20). The hydrostatic intravesical pressure ranges between 4 and 28mm Hg in normal supine patients, the pressure being dependent on the volume within the bladder and rising with increasing volume up to 350ml. Mean intravesical pressures were found higher in obstructed bladders than in normal bladders at each volume, confirming a previous observation of Claridge and Shuttleworth (27). (Fig. 5) Changing from supine to erect position increases the intravesical resting pressure. The mean bladder volume in patients with obstructed bladders was less (254ml) than in those patients with non-obstructed bladders. This change in bladder volume could not be related to residual urine as residual urine was not a constant factor in these patients. Whether this smaller mean volume in obstructed bladders can be related to the observation of Osius and Hinman who found intramural tensions higher than normal in small bladders that had undergone acute retention is unknown (28).

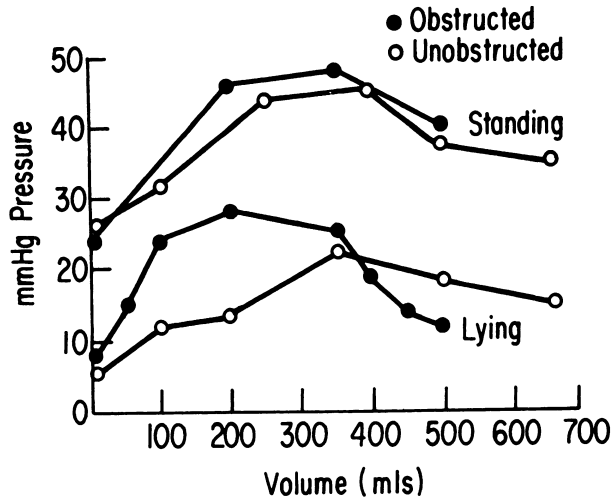

FIGURE 5. Mean intravesical resting pressures in patients standing and supine as a function of bladder volume. From Lenaghan. J. Urol. 103: 320–326, 1970.

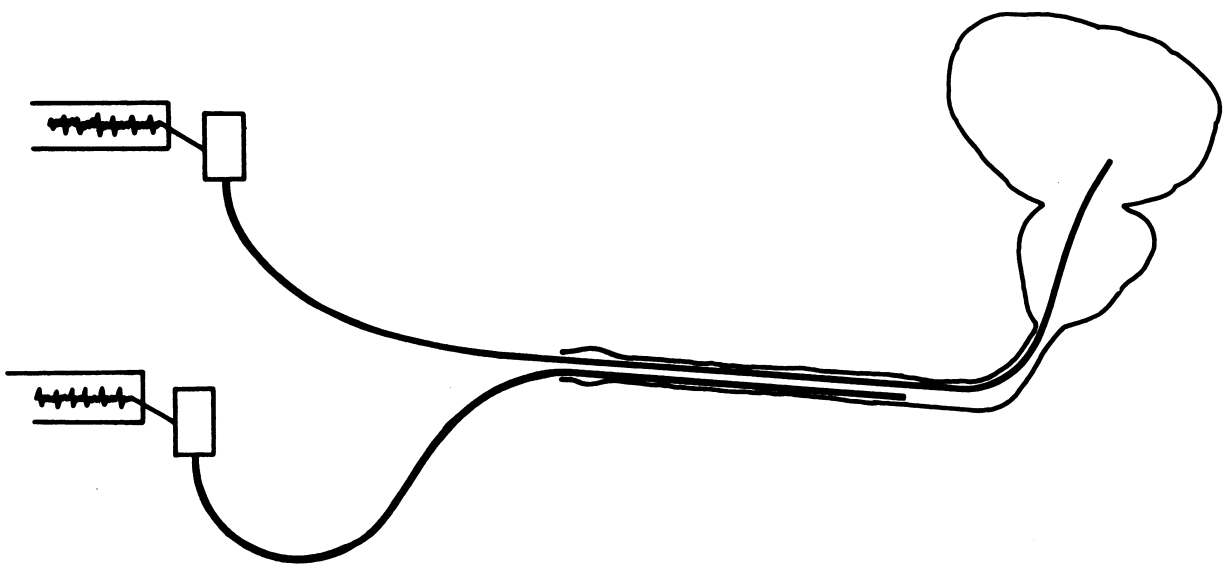

FIGURE 4. Demonstrates placement of pressure catheter for determining prostatic urethral resistance.

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Lenaghan's studies did refute the belief that an enlarged prostate could be present without causing obstruction. In all patients, when endoscopy showed enlarged prostatic lobes meeting in the midline, the resistance to flow across the prostatic urethra was increased. However, the degree of resistance to flow could not be related to prostatic size as determined by visual or digital examination.

In summary, peak and mean urinary flow measurements are different in obstructed and nonobstructed bladders and provide an objective method for assay of the clinical symptom complex of decrease in size and force of the urinary stream hesitancy, urgency and prolonged voiding. Simultaneous measurement of intravesical and intraurethral pressure during voiding provides an index of the pressure gradient across the occluding prostatic urethra and is perhaps the most meaningful index of the degree of outflow occlusion. Unfortunately, few of the objective measurements can be related to the size of the occluding prostate, and none of the objective measurements can be used to predict the severity of the clinical symptomatology which the patient will experience.

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DISCUSSION

J. E. McNeal: Is there any conclusive evidence that the size of BPH nodules in patients with clinical obstruction is larger than in people in the same age group without clinical obstruction?

D. F. Paulson: Are you asking whether size plays a role in the symptomatology which patients present?

McNeal: No. Does size play enough of a role that there is a clear separation in size ranges between those with obstruction and those without?

Paulson: The answer to your question is "yes" for those patients who have physiologically demonstrable obstruction, but size and symptomatology do not correlate in all men. Some patients have large glands and void relatively well or do not void well yet are content and not interested in having anything done. Other patients with small glands are symptomatic and desparate for relief from their symptomatology.

McNeal: An extreme degree of obstruction is frequently produced by small mid-line nodules at the bladder neck. It may be only a few grams of tissue. On the other hand, there are large nodules laterally with no trabeculation of the bladder, suggesting that obstruction has not been significant.

Paulson: Isolated mid-line lobes are not the most frequent clinical presentation.

L.M. Franks: I would confirm that. If one compares surgically removed and autopsy material, no differences are apparent. Perhaps it might be worth mentioning that we are all talking about BPH. In fact, a lot of urinary obstruction is not related directly to BPH but to bladder neck fibrosis and so on.

Paulson: You are quite right.

W. W. Scott: I would like to ask Dr. Franks for the evidence that the sizes are the same, because in the man who under goes an enucleation procedure, all that is left is the outer prostate, whereas the man who comes to autopsy with benign hyperplasia has his entire prostate.

Franks: Yes, but if you compare the benign nodules removed and the benign nodules in the autopsy series, one can get quite a fair comparison.

J. T. Grayhack: There is a well-documented group of observations starting with French urologists and including Cabot documenting that the small prostate often resulted in the most serious bladder neck obstruction phenomenon.

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RESULTS OF SURGICAL TREATMENT OF BENIGN PROSTATIC HYPERPLASIA

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The ultimate goal of this workshop is to stimulate efforts to prevent or control the development of benign prostatic hyperplasia and its sequellae on the urinary tract of man. Acceptance of future efforts to achieve these goals should depend on their effectiveness and risks as compared to procedures used for this purpose today. Available evidence indicates that castration effectively prevents BPH. Although this procedure was used as a therapeutic measure in patients with bladder neck obstruction at the turn of the century (7,55,56), it was abandoned in favor of excision of the obstructing tissue. There is little likelihood that castration would be acceptable as a prophylatic procedure today even if a high probability of significant bladder neck obstruction secondary to BPH could be predicted in an individual patient. The accepted definitive current therapeutic approach to relieve significant bladder neck obstruction from BPH is prostatectomy. A brief review of the current status of the surgical procedures used for this purpose is indicated.

Indications For Prostatectomy

Since the vast majority of men over 60 (16,37) have definite evidence of benign prostatic hyperplasia on gross or microscopic examination of the prostate, few, if any, clinicians would suggest that the mere presence or even the mass of this pathophysiologic change was an indication for surgical treatment. Instead the presence and degree of bladder neck obstruction and the response of the urinary bladder to it are usually utilized to select patients in whom surgical removal of BPH is thought to be desirable. The clinician has learned that the development of a small amount of hyperplastic tissue in the form of a bar or circumferential narrowing at the bladder neck can often produce serious symptomatic sequellae while a much more massive amount of hyperplasia located more distally in the prostatic urethra may produce little clinically significant change. Therefore, objective evidence of a mechanical impairment to emptying the bladder plays a major role in evaluating patients with BPH (see Paulson, this symposium, p 119). Cystoscopic examination identifies the presence of obstruction and of bladder changes such as trabeculation and diverticula secondary to it. Determination of residual urine is utilized to assist in recognizing bladder decompensation. The presence of renal failure, infection, or calculi on the basis of stasis related to obstruction is usually a definite indication for the relief of bladder neck obstruction and so is acute persistent urinary retention. The degree of symptomatic discomfort as manifested by nocturia, increased diurnal frequency, hesitancy, intermittency, etc., that a patient and his physician think should be tolerated when accompanied by objective evidence of obstructing BPH is highly variable but is also an accepted common indication for prostatectomy, Lytton et al (31) calculate that with current practices, there is a 10% probability that a man who is 40 will require a prostatectomy if he lives to 80 years of age.

Operative Procedures

Sporadic accidental or perhaps purposeful removal of portions of the prostate occurred with surgical adventures in the perineum since the mid-

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dle ages or earlier (25). Planned purposeful removal of the benign adenomatous portion of the prostate became a regular part of surgical therapy toward the end of the 19th century. Recognition that clinically important bladder neck obstruction could result from a variety of gross and microscopic pathologic changes in the bladder neck-posterior urethral area led to the development of increasingly sophisticated, accurate diagnostic tools and rational use of effective surgical procedures with minimal risk. Experience has shown that essentially complete removal of the tissue undergoing hyperplasia is necessary to achieve satisfactory relief of bladder neck obstruction; at the present time, each of the procedures regularly employed in the surgical treatment of BPH permits achievement of this goal when properly utilized. Currently, two of the common operative approaches to BPH employ a suprapubic incision (Fig. 1); in one (suprapubic prostatectomy), the nodular hyperplasia is removed through the bladder and in the other (retropubic prostatectomy), the adenomectomy is carried out by an incision through the ventral aspect of the compressed normal tissue, the surgical capsule. One procedure (perineal prostatectomy) is carried out in the extreme dorsal lithotomy position utilizing a perineal incision to permit an approach to the adenoma through the dorsal or posterior aspect of the surgical capsule. The most common surgical procedure used today employs an endoscopic instrument passed through the urethra to remove the hyperplastic tissue in a piecemeal fashion (transurethral prostatectomy). A variety of other surgical approaches has been used sporadically with apparent successful relief of obstruction of the bladder neck due to BPH but none has enjoyed common prolonged acceptance.

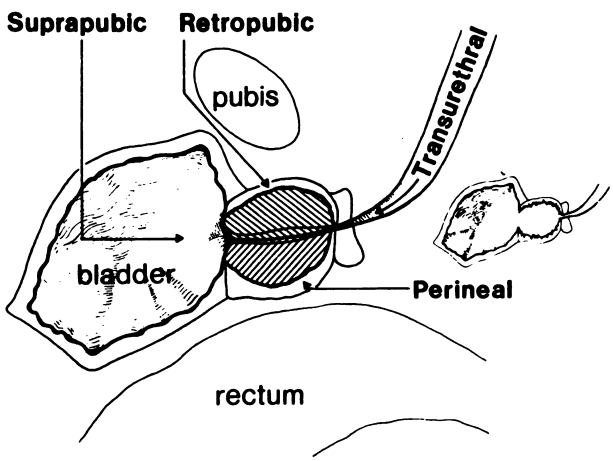

FIGURE 1. Schematic diagram to illustrate the common operative procedures employed to excise benign prostatic hyperplasia. The so-called surgical capsule of the prostate, the compressed prostatic tissue remaining after excision of BPH by any route, can be seen bridging between the bladder neck and the external urethral sphincter in the insert.

Prostatectomy is carried out to relieve bladder neck obstruction so that serious abnormalities secondary to it such as renal failure, urinary tract infection, or stone are eliminated or controlled and so that obstructive symptoms will be relieved. Coupling an improved diagnostic ability with advances in pre- and post-operative care, anesthesia, and surgical technique has allowed a continuing improvement in the results obtained. The opportunity for surgical relief of bladder neck obstruction has been extended to increasingly poor risk patients with decreasing mortality and morbidity. A review of the results achieved is desirable.

Mortality Rate

There seems little doubt that there has been a progressive decrease in mortality (Fig. 2) and in serious complications since prostatectomy became an accepted approach to management of bladder neck obstruction from BPH at the turn of the century. For the most part, mortality rates have been reported for specific procedures. At one time the risk of suprapubic prostatectomy was consistently greater than that associated with perineal prostatectomy (Table I). However, the rates for open prostatectomy now seem essentially comparable, with risk of mortality fluctuating around 1%; the risk of death is less in patients subjected to transurethral resection so that survival in all patients subjected to prostatectomy usually exceed 99% (47).

Review of the cause of death in recent series (Table II) indicates that technical errors and abnormalities directly related to the surgical procedures are no longer commonly identifiable. Patients with increased risk can be recognized to some degree preoperatively. For example, individuals with recognized renal failure have traditionally been regarded as poor risks for prostatectomy and recent observations tend to support this prejudice (35). Similarly, older men have a lessened chance of survival (2,5,31,36,48); for example in the recent review of their experience with transurethral resection in 2223 patients, the University of Kansas Medical Center noted an overall mortality of 1.3% compared to 3.5% in patients over eighty (34). This group (34) found a correlation between increased

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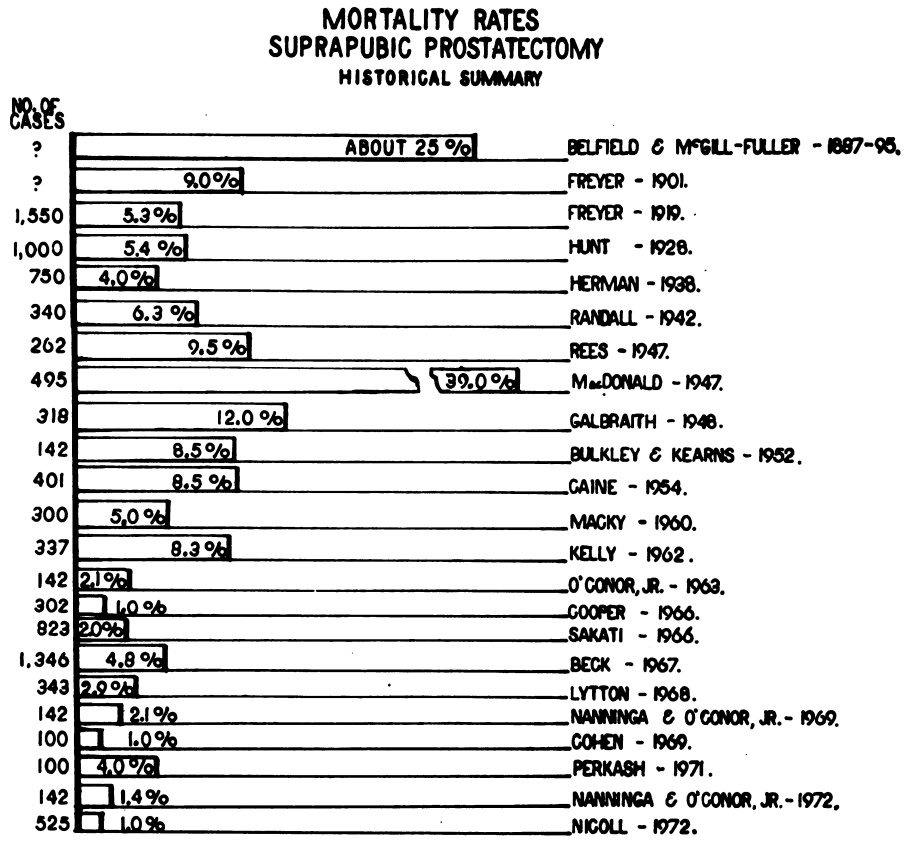

FIGURE 2. Decreasing trend of mortality after suprapubic prostatectomy.

TABLE I—Reported Mortality Rates in Prostatic Surgery

Author	*Location*	*Date*	*Number of Patients*	*Mortality Rate*
Perineal Prostatectomy				
H. Young (57)	Baltimore	1926	1000	3.2%
D. Higbee (22)	Denver	1951	572	1.7%
Davis & Lee (12)	Omaha	1953	2050	2.9%
Turner & Belt (53)	Los Angeles	1957	1694	2.9%
Sakati (47)	New York	1966	131	0.8%
Lilien (30)	Syracuse	1968	150	0%
Lytton (31)	New Haven	1968	83	4.8%
Suprapubic Prostatectomy				
Freyer (20)	England	1919	1550	5.3%
Hunt (26)	Rochester	1928	1000	5.4%
Herman (20)	Philadelphia	1938	750	4.0%
Randall (12)	Philadelphia	1942	340	6.3%
Rees (46)	England	1947	262	9.5%
Mac Donald (11)	Canada	1947	495	39.0%
Galbraith (17)	England	1948	318	12.0%
Bulkley & Kearns (6)	Chicago	1952	142	8.5%
Caine (8)	London	1954	401	8.5%
Macky (32)	New Zealand	1960	300	5.0%
Kelly (29)	Dearborn, Mich.	1962	337	8.3%
O'Conor (43)	Chicago	1963	142	2.1%
Cooper (10)	Los Angeles	1966	302	1.0%
Sakati (47)	New York	1966	823	2.0%

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TABLE I—Reported Mortality Rates in Prostatic Surgery—Continued

Author	*Location*	*Date*	*Number of Patients*	*Mortality Rate*
Beck (3)	New Zealand	1967	1346	4.8%
Lytton (31)	New Haven	1968	343	2.9%
Nanninga & O'Conor (39)	Chicago	1969	142	2.1%
Cohen (9)	Baltimore	1969	100	1.0%
Perkash (45)	India	1971	100	4.0%
Nanninga & O'Conor (38)	Chicago	1972	142	1.4%
Nicoll (42)	Escondido, Ca.	1972	525	1.0%
		Retropubic Prostatectomy		
Millin (36)	England	1949	757	4.5%
Jacobs (28)	Glasgow	1951	500	6.0%
Newman (41)	Australia	1951	103	8.0%
Ostenfeld (44)	Denmark	1951	114	4.3%
Caine (8)	London	1954	207	6.8%
Salvaris (48)	England	1960	1200	4.2%
Stearns (50)	Boston	1961	500	0.6%
Allan and Coorey (2)	England	1966	1000	5.2%
Sakati (47)	New York	1966	276	0.7%
Lytton (31)	New Haven	1968	64	0%
Salvatierra (49)	Pomona, Ca.	1972	157	0%
		Transurethral Resection		
Alcock (1)	Iowa City	1935	600	4.6%
Kretchmer (20)	Chicago	1935	551	3.2%
Cook County Hosp. (20)	Chicago	1937	283	18.5%
Herman (20)	Philadelphia	1938	500	2.6%
Randall (12)	Philadelphia	1942	315	2.2%
Nesbit (11)	Ann Arbor	1946	2425	1.8%
Univ. of Minn. (11)	Minneapolis	1950	1560	1.2%
Nesbit (40)	Ann Arbor	1950	675	1.5%
Mayo Clinic (Punch) (11)	Rochester	1950	11,522	1.2%
Hayward (19)	Orlando	1950	470	1.5%
Lee (12)	Omaha	1954	794	1.7%
Bergman (5)	Los Angeles	1955	1000	1.8%
Holtgrewe (23)	Kansas	1962	2015	2.5%
Sakati (47)	New York	1966	2473	0.6%
Lytton (31)	New Haven	1968	271	1.1%
Melchior (34)	Kansas	1974	2223	1.3%
Ignatoff (27)	Chicago	1974	681	0.3%

risk and removal of a large adenoma (60+ gms); others have also noted a greater risk in males with large glands (5). These observations provide evidence that selection of low risk patients for operation and employment of permanent catheter drainage in high risk individuals could be an explanation for the progressive decrease in mortality noted with prostatectomy. Although the reported rejection rate for prostatectomy has remained relatively constant (Table III), most experienced urologists have the impression that, with improvement in operative techniques and perioperative care, they have advised older individuals with increasingly numerous and severe respiratory, renal, cardiovascular, and other complicating conditions to accept the risk of prostatectomy rather than the inconvenience of a catheter life (39). Davis & Lee (12) point out that this practice over a long span of time has been a cause for a stable or slightly increased mortality following perineal prostatectomy in their experience. When one reviews the indications for prostatectomy (under 70 years of age, normal kidneys, fairly healthy bladder, marked enlargement of the prostate on rectal, decided urethral impediment, over 4 ounces of residual urine, symptoms of frequency, pain, tenesmus, and burning, and attacks of urethral fever, epididymitis and hematuria) as compared to these for use of an indwelling catheter (any age over 50, considerably diseased kidneys, atonic bladder, any size prostate, catheterization possible without difficulty or complication, over four ounces of residual urine, heart feeble, artery disease, and symptoms similar to individuals suitable for prostatectomy) attributed

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TABLE II—Causes of Mortality in Prostatectomy

	Transurethral Resection Mortality University of Kansas (23) (34)		*Davis & Lee Perineal Prostatectomy Mortality (12)*
	1955–60 (2015 Cases)	*1965–71 (2223 Cases)*	*1927–53 (2050 Cases)*
Myocardial Infarct	0.9%	0.5%	0.3%
Pulmonary Embolus	0.5	0.1	0.7
Cerebrovascular Accident	0.2		0.4
Pyelonephritis & Sepsis	0.2	0.1	0.7
Pneumonia	0.2		0.05
Hemorrhage	0.1		0.3
Acute Renal Failure	0.1	0.1	0.15
G.I. Bleeding	--	0.2	0.05
Congestive Heart Failure	--	0.2	--
Respiration Failure	--	0.1	--
Ruptured Aneurysm	0.1	0.1	--
Mesenteric Artery Thrombosis	0.05	0.1	--
Cerebral Arteriosclerosis	--		0.05
Femoral Artery Embolus	--		0.05
Perforation of Colon	--		0.05
Intestinal Obstruction	--		0.05
Parkinson	--		0.5
Total	2.5%	1.3%	2.9%

to C.H. Mayo in 1902 (33), there is little doubt that current practice eliminates the need for a catheter life in many patients formerly advised to accept it. Patient selection for prostatectomy seems unlikely to be the cause of the reduction of mortality rate noted. On the other hand, selection of the type of prostatectomy and patient preparation for it seems likely to be a significant factor. The possibility that various arbitrary designations of cause of death as related or unrelated to prostatectomy may have distorted the results noted seems to warrant little serious consideration in view of the broad data base available for review.

Incontinence

The incidence of involuntary loss of urine in the unoperated adult male without neurologic disease is not documented. Certainly, the use of some type of protective pad to guard against urgency, incontinence and terminal dribbling occurs with increasing frequency as the human male ages. These types of incontinence can often be improved by operative removal of the obstructing prostate; they can also be aggravated. Furthermore, men who have had no recognizable difficulty with urinary control may develop total incontinence or such severe urgency or stress incontinence as to require a device to assure continued social acceptability. Table IV lists the incidence of incontinence reported in experiences with the common types of prostatectomy. The need to use a device to achieve urinary control is an undesirable sequelae of prostatectomy.

Potency

Persistence of normal sexual ability is difficult to document. Factors in failure of normal functions are difficult to identify and assess. In addition to such organic causes as impaired circulation, neurologic

TABLE III—Operability Rate in Prostatectomy

Author	*Date*	*% of Patients Operable*
Harris (18)	1934	97%
Stewart (51)	1945	86%
Hey (21)	1945	98%
Jacobs (28)	1951	97%
Ellis & Leatherdale (14)	1958	94%
Tagert (52)	1960	97%
Allan & Coorey (2)	1966	90%
Lytton (31)	1968	92%
Beck (3)	1970	97%

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TABLE IV—Incidence of Incontinence

Procedure	*Date*	*Number of Patients*	*Incontinence Rate*
Perineal			
Young (57)	1926	450	0.7%
Turner & Belt (53)	1957	1964	1.9
Lilien et al (30)	1968	150	†6/14
Suprapubic			
Caine (8)	1954	401	0.7
O'Conor, Bulkley & Sokol (43)	1963	374	0
Cooper & Ashamalla (10)	1966	302	0
*Beck (4)	1970	161	1.2
Retropubic			
Millin et al (36)	1949	724	0.3
Caine (8)	1954	207	0.5
Stearns (50)	1961	500	0.4
Allan & Coorey (2)	1966	1000	0.6
Salvatierra (49)	1972	157	0
Transurethral			
Hayward et al (19)	1950	470	1.3
Bergman et al (5)	1955	1000	0.5
Holtgrewe & Valk (24)	1964	840	†1.4/5.5
Ignatoff (27)	1974	681	0.4

*Hryntshak Technique
†device required/no device

and endocrine deficit and perhaps accessory sex gland injury, psychological factors play a major role in the loss of normal sexual function. There seems little doubt that some patients who have had sexual intercourse prior to prostatectomy, no longer function sexually following the operation. Caine (8) reported 15 (4%) of 370 patients subjected to a variety of surgical procedures to remove their prostatic adenoma felt the operation had improved their sexual function and 171 (46%) felt the procedure had a deleterious effect; 184 (50%) reported no effect on sexual function. Stearns (50) reported that 228 of 280 potent patients subjected to retropubic prostatectomy claimed retention of function postoperatively. Generally the transurethral, suprapubic, and retropubic operations have been thought to interfere with sexual function only occasionally whereas the perineal procedure has a particularly bad reputation in this regard. This pessimism seems justified on superficial inspection of the data presented by Lilien et al (30) (Table V). However these authors emphasize that they selected the high risk debilitated patient for perineal prostatectomy and the more vigorous individual who was concerned about retention of sexual function for the suprapubic procedure. The data gathered by Finkle and Prian (15) in a prospective study of the effect of perineal, suprapubic and transurethral prostatectomy indicates retention of sexual function by most patients and tends to minimize the importance of the operative procedure. These authors concluded that the presence of a healthy willing partner was a most important factor in maintenance of the ability to have sexual intercourse. Many patients have some form of sexual dysfunction postoperatively. Retrograde ejaculation is the most common of these and has been reported in most patients subjected to open prostatectomy (8); this phenomenon has been reported in 30% (8) to almost all (34) patients subjected to transurethral resection. This variation may well be a reflection of the resection technique employed. If the nature of this phenomenon is or has been explained, it rarely causes any lasting concern.

Stricture

Inappropriate healing at the bladder neck or circumferential scarring along the urethra can result in a troublesome narrowing of the urethral lumen.

TABLE V—Potency Status Following Prostatectomy for BPH

	Lilien et al (30)		*Finkle and Prian (15)*	
Perineal	33/103 [1]	(26%) [2]	24/35	(71%)
Suprapubic	34/54	(61%)	22/35	(87%)
Transurethral	--		23/32	(95%)

[1] Potent pre-op/total number of patients; [2]( ) per cent of patients retaining pre-operative potency. The case numbers for Lilien et al (30) were inconsistent but the potential variation did not affect the results appreciably.

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At times, strictures are easily controlled and result in a very limited patient discomfort; at other times, they are virtually uncontrollable by simple techniques and cause such serious secondary changes in the urinary tract that they are treated by complex staged operative procedures. Reported incidence of stricture following prostatectomy varies (Table VI). A portion of the variation probably is due to patient selection and technical factors but the duration and nature of patient follow-up also plays a role. The amount of serious disability resulting from a urethral stricture following prostatectomy is not clear; most urologists have the undocumented impression that serious stricture problems are becoming much less common as intruments, catheters, means of controlling infection and skills improve.

Recurrence

The incidence of recurrent benign prostatic hyperplasia following prostatectomy is not recorded but it is probably high. Weyrach (54) estimated that only 2% of patients require reoperation for recurrence of BPH and that the incidence is higher in younger patients. The occurence of adenoma producing sufficient obstructing changes to require a repeat excision is recorded in several series (Table VII); this varies from 0.3% after perineal prostatectomy to 7% after transurethral resection. While these figures probably reflect the nature of the original pathologic process, the completeness of follow-up, and indications for reoperation as well as the development of recurrent obstructing tissue, they do seem to indicate a definite difference between open and endoscopic techniques for removal of the original BPH. The reported series of patients subjected to transurethral resection frequently include patients with carcinoma; in addition, the type of pathologic configuration preferentially treated by transurethral resection, e.g., bladder neck contracture, has a recognized tendency to recur whatever treatment route is utilized. Despite these considerations the

TABLE VI— Incidence of Urethral Stricture in Prostatectomy

Procedure	*Date*	*Number of Patients*	*Urethral Stricture*
Perineal			
Turner & Belt (53)	1957	1694	3.9%
Suprapubic			
Caine (8)	1954	401	5.1
*Beck (4)	1970	161	11.2
Retropubic			
Millin (36)	1949	675	0.4
Caine (8)	1954	207	14.9 (10.1)**
Allan & Coorey (2)	1966	1000	2.1 (1.3) Temporary 0.3 Persistent
Transurethral			
Hayward et al (19)	1950	470	16.2 (10)
Bergman et al (5)	1955	1000	5.1 (1.7)
Holtgrewe & Valk (24)	1964	800	2.7

*Hryntshak Technique
**( ) Meatal—Fossa navicularis stricture

TABLE VII—Reoperation Following Prostatectomy for BPH

Procedure	*Date*	*Number of Patients*	*Reoperation*
Perineal			
Turner & Belt (53)	1957	1694	0.3%
Suprapubic			
Bulkley & Kearns (6)	1952	142	0.7 [1]
Nanninga & O'Conor (38)	1972	142	0
Retropubic			
Stearns (50)	1961	500	1.6
Allan & Coorey (2)	1966	1000	0.4
Transurethral			
Bergman et al (5)	1955	1000	5.3
Holtgrewe & Valk (24)	1964	840	6.4
Ignatoff (27)	1974	681	7.0

[1] In the original suprapubic series of the O'Conor group (6) 1 patient, 0.7% was operated for recurrent adenoma; 8 patients required operation for bladder neck contracture. In the second series (43) 5 patients were operated for bladder neck contracture and this complication was not reported in the last 2 series (38,39).

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data indicating a higher rate of repeat operation after transurethral resection seem to indicate a significant role for incompletely removed hyperplastic tissue in this phenomenon.

Acute Morbidity

In contrast to mortality, the recorded morbidity has shown a tendency to stabilize at a rate between 15 and 20% (Table VIII). Technical errors constitute a small cause for morbidity; for instance, in the transurethral resection series from the University of Kansas, extravasation occurred in 1.1% and 0.6% of the cases in the 1955–1960 and 1965–1971 series, respectively. Procedures to stop secondary bleeding were 0.5 and 1.1% in each series, respectively. The secondary resection rate was 3.4 and 0.9%. Epididymitis, pyelonephritis, and uremia were major factors in the morbidity; pneumonia, non specific sepsis, ileus, thrombophlebitis and mental confusion comprise the remaining major cause of morbidity in this series.

Conclusions

Prostatectomy is the accepted procedure at this time for relieving bladder neck obstruction from benign prostatic hyperplasia. Many patients with minimal symptoms and secondary changes from their obstruction are advised to tolerate their limited disability. When patients have enough bladder neck obstruction to produce severe symptoms and urinary stasis with its sequelae and are not prohibitive operative risks, excision of the obstructing BPH is usually advised by the suprapubic, retropubic, perineal, or transurethral route. The current mortality rate for these procedures is less than 1%, even though poor risk individuals so common in this group of patients are rarely denied operation in these days. Short term morbidity is appreciable but long term serious disability is limited. The procedure provides rapid relief of symptoms and correction of urinary stasis in approximately 90–98% of the patients operated on (2,4,13,24). Most patients retain potency. The need for further operative treatment is uncommon. New approaches to the management of bladder neck obstruction due to BPH should provide at least comparable results.

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TABLE VIII—Morbidity Associated with Prostatectomy for BPH

	Suprapubic Prostatectomy O'Conor Series (6,43,38,39)				*Transurethral Prostatectomy Valk Series (23,34)*	
Years	1942–50	53–61	61–65	66–70	1955–60	65–71
Cases	142	142	142	142	2015	2223
Hemorrhage	3.5%	1.4%	3.5%	4.2%	0.5%	2.3%
Myocardial Infarct	1.4	0.7	0.7	0.7	0.2	0.8
Congestive Failure	2.1	0	0	1.4	0.4	0.2
Pulmonary Embolism	3.5	0	0	0.7	0.3	0.2
Renal Failure	0	0.7	0	0	0.7	0.3
Epididymitis	2.1	4.9	3.5	X	6.0	2.6
Pyelonephritis	2.8	0.7	0	X	1.1	0.4
Pneumonia	2.8	0.7	0.7	2.8	1.3	0.7
Thrombophlebitis	0	1.4	0	X	0.7	0.4
Endotoxin Shock	X	0	1.4	0	0.5	X
G. I. Bleeding	0	0	0.7	1.4	0.2	0.3
Extravasation	--	--	--	--	1.1	0.6
Suprapubic Fistula	2.1	0	0	X	--	--
Secondary Resection	--	--	--	--	3.4	0.9
Total [1]	23.2%	11.9%	14.1%	14.1%	18%	17.3%

X–Not listed; –not pertinent

[1] Totals include unlisted complication such as wound infection (0–2.8%), and partial incontinence (0–2.1%) in the suprapubic series and fluid absorption toxicity (0.4–0.9%), and "fibrinolysis" (0.3–0.9%) in the transurethral series. The totals listed for the suprapubic series were calculated by assuming no patient had more than one complication.

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DISCUSSION

J. D. WILSON: In this country, what is the frequency of transurethral resection *vs*. the other procedures.

J. T. GRAYHACK: It is approximately 80%.

H. BRENDLER: I'm interested in the last figures you quoted. Did you mean that of ten people with clinical BPH, one has surgery and 8 or 9 walk around with clinical BPH?

GRAYHACK: Yes. A person, who at the age of 45 would not get up at night and would have no hesitancy or intermittancy and who at the age of 55 might get up once or twice a night, have to wait an extra second or two to void, and not have any more severe complaints, would have approximately a 10% chance of being subjected eventually to a surgical procedures. The data on which this is based is difficult to defend, but the statement corresponds to my impression.

BRENDLER: What is the source of the figures:

GRAYHACK: Lytton calculated the figure from the New Haven experience. I happen to agree with it.

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BENIGN PROSTATIC HYPERPLASIA: CURRENT STATUS OF MEDICAL TREATMENT

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Initially, it is important to define what is meant by "medical treatment" of benign prostatic hyperplasia. Brendler has already discussed the natural history of BPH (this publication, page 101), and Paulson has described the evaluation and assessment of bladder neck obstruction (this publication, page 119); both presentations provide important background material for this discussion.

It is difficult to determine who first initiated any form of medical management of bladder outlet obstruction. It seems clear that prior to the advent of prostatectomy for benign enlargement, or some form of surgical therapy for bladder neck contracture, the main forms of therapy involved urinary diversion by catheter, either intermittently or continuously, and irrigations of the catheter with agents which hopefully were antiseptic.

Most urologists appreciate that benign nodular hyperplasia prostates—the apparent cause of acute or chronic urinary retention in most men—may become large, even huge, and yet not result in outlet obstruction. Yet, it is reasonable to speculate that the basic cause of outlet obstruction is the prostatic enlargement. Recently, Valk (1) stated that approximately 70% of his patients who required transurethral resection for outlet obstruction had clear-cut benign hyperplasia in gross and microscopic examination of the tissue removed. Another 15% suffered contracture of the vesical neck, the cause of obstruction in the remaining 15% being due to prostatic cancer. The excellent functional results following surgical removal of the benign enlargement where the "inner prostate" is literally shelled-out from the "outer or true prostate" attest to the importance of removing this benign tissue in the relief of outlet obstruction, as Doctors Grayhack and Sadlowski have described (this publication page 125).

Consequently, in this discussion of the current status of medical treatment, we have chosen to limit our remarks mainly to efforts to shrink the nodular hyperplasia mass by so-called hormonal manipulations.

At the outset, many of us are fully aware of the difficulties involved in the evaluation of what we have tried to accomplish by medical treatment; namely, the alleviation of the symptoms of outlet obstruction, the prevention or reversal of the results of obstruction such as urinary retention and resultant uremia, and a significant reduction in the size of the benign, enlarged inner prostate. Those working in this area are fully aware, too, of the admonitions of authors, such as Roscoe Clarke (2), and many others, that control series are necessary and that, "Conclusions as to the efficacy of endocrine or other new methods of treatment are only

This work was supported in part by a Training Grant from the National Institute of Arthritis, Metabolism and Digestive Diseases, of the National Institutes of Health, Bethesda, Maryland. (AM 05674–14).

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valid if cases are followed-up for a considerable period of time." Clarke suggests five years.

A chronologic historical review of the multitudinous efforts made to control prostatic hyperplasia seems less important than examining current efforts to do this, in light of an appreciation of rigid protocols, involving prospective studies, randomization of patients, placebos, cross-overs, biopsies, etc.

David Paulson and Richard Kane of Duke University Medical Center and the Veterans Administration Hospital at Durham presented the most recent study of this nature (3). They utilized an excellent protocol which has been presented in brief and should be made available in its entirety for future efforts. Basically, "patients were considered candidates for the study if symptoms were of sufficient clinical severity to cause them to seek treatment but did not require immediate surgical intervention or if a past history of severe cardiovascular or respiratory disease presented great surgical hazard. All patients were candidates for surgical prostatectomy but chose to enter the study of their own volition." Cystoscopy and prostatic biopsy were carried out initially. The patients recorded their symptoms in a diary and peak and mean urine flows were determined at three week intervals during a six week placebo baseline period. Residual urine and rectal evaluation of the prostate was also carried out at these times. After completion of the baseline studies, the patient was assigned to a treatment or placebo group for 15 weeks with continuing evaluation at three week intervals. Following completion of medical treatment, the patients were followed for 12 weeks with reversal of placebo and drug groups.

Medrogestrone was chosen as the test agent in the trial because of the favorable experiments of Rangno (4) with this progestational steroid and the earlier experiences of Geller et al (5,6) and Scott and Wade (7) indicating promise for progestational agents in the treatment of benign prostatic hyperplasia in men. The so-called "antiandrogenic" properties of the progestational steroids lead to their choice. Medrogestrone was given for 15 weeks at either a 7.5 or a 15 mg. twice daily dose in the study reported by Paulson and Kane. A group of patients received a placebo for the same period.

In brief, 19 of 23 patients completed the study. The authors concluded, "In the present study, no significant difference in symptomatic improvement, clinically determined prostatic size, or residual urine were found when patients were treated with medrogestrone or placebo. Patients treated with 15 mg. medrogestrone twice a day showed a significant increase in peak urine flow, mean urine flow, and total volume of urine voided during micturition. Use of 7.5 mg. medrogestrone twice a day did not seem to offer any advantage over placebo."

On October 31, 1974, at a conference on benign prostatic hyperplasia sponsored by the Merck Sharp and Dohme Research Laboratories, several of us here today learned from Doctor Rangno that reanalysis of these data revealed no significant difference in any parameter, including peak urine flow, mean urine flow and total volume of urine voided at any one time. This was a disappointment, indeed, and may have been due to too low a dosage. At the same conference, Doctor Rangno emphasized the importance of making histologic examinations of biopsy materials before treatment and at 24 and 48 weeks. Such observations were not reported in the study by Paulson and Kane (3), but were in the earlier study by Rangno et al (4). Rangno stated that he was convinced in his own mind that if medrogestone is given in a dosage of 100 mg/day to patients with nodular hyperplasia, one will invariably observe changes in the acini and its epithelium, as long as the patient is on the drug. Their report of such changes follows: "Striking constant histologic changes were observed after medrogestone therapy. There was absence of acinar fronds. The epithelium was flat and broken and lytic fragments filled the lumen. There was no consistent change in the stroma or excretory ducts. In those patients who received medrogestone during the first 24 weeks, we observed a complete reversal of the lytic changes to normal by the end of the placebo period (24 weeks) which followed."

Earlier, reference was made to our own studies with cyproterone acetate (7) and the studies of Geller and his associates (5,6) with other progestational agents. We appreciate that our study was only an exploratory one, devoid of controls. However, it was carefully conducted in terms of patient selection, urine flow rates, residual urine determinations, measurement of serum testosterone levels, and assessment of histologic changes in the adenomas during administration of cyproterone acetate. As noted above, the importance of the latter observations was emphasized strongly by Rangno and his associates (4) in their subsequent studies.

At the time of our report in 1968, 13 patients with significant benign prostatic obstruction had received 50 mg cyproterone acetate orally daily for periods ranging to 15 months. Urinary obstructive symptoms were judged to have lessened in 11 pa-

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tients, remained unchanged in one and worsened in one. Prostate size, estimated by digital palpation, decreased in 7 patients and remained unchanged in 6. Urine flow rates increased in nine patients, remained essentially the same in two and were reduced in two (Fig. 1). Measured volumes of residual urine decreased in eight patients, remained the same in two and increased in three (Fig. 2). All but one patient showed a fall in serum testosterone during treatment (Fig. 3). This patient probably did not take the drug regularly and was one of three patients who showed an increase in prostatic epithelial height.

Prostatic tissue was obtained by needle biopsy in all patients before and during treatment, the time interval being close to 60 days. The average epithelial height in these specimens was measured by Doctor William Shelley, without his knowing the identity or clinical state of any of the patients (Fig. 4). There was insufficient epithelium to permit measurement in 2 of 26 specimens, leaving 11 patients in whom a comparison could be made. Epithelial height decreased in eight patients and increased in three patients. Figs. 5A and B illustrate the decrease in acinar epithelial height in the patient who was judged to have shown the greatest decrease. This patient actually showed a decrease in urine flow rates with treatment, essentially no change in his residual urine and only a modest decrease in serum testosterone levels. Again, we appreciate and emphasize that this study lacked the necessary controls.

As indicated heretofore, Geller and his associates (5,6), reported on the use of progestational agents in the treatment of benign hyperplasia in 1965 and 1969. In the earlier series of 10 patients, hydroxyprogesterone caproate was given biweekly in a dosage of 1.5 gm intramuscularly. Two patients received dexamethasone in addition. This earlier series lacked controls. In the later series of 11 patients, eight were treated with hydroxyprogesterone caproate and three with chlormadinone acetate. The revised protocol for this study provided better controls, but not to the extent of the protocol

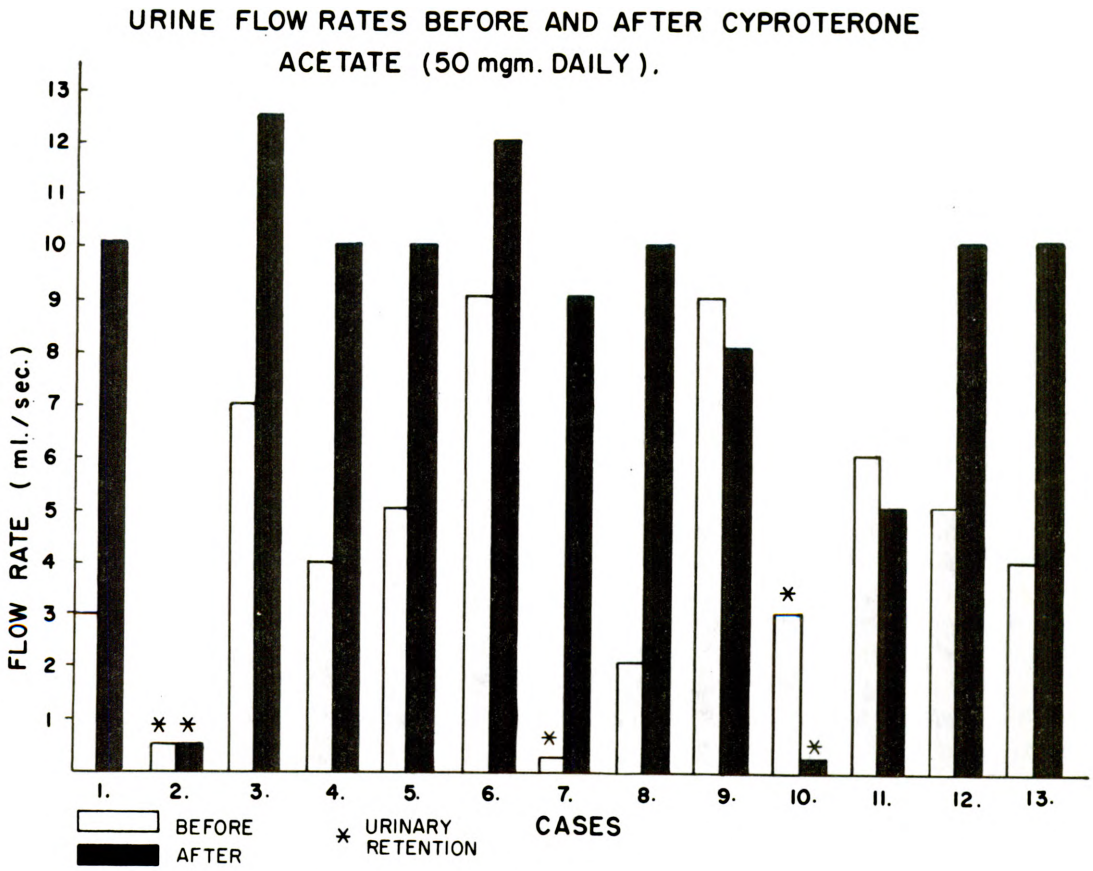

FIGURE 1. Urine flow rates increased in 9 of 13 patients. Patients 2 and 10 required surgical relief 4 and 2½ months after initiating drug therapy. From Scott, W. W., and Wade, J. C. (7)

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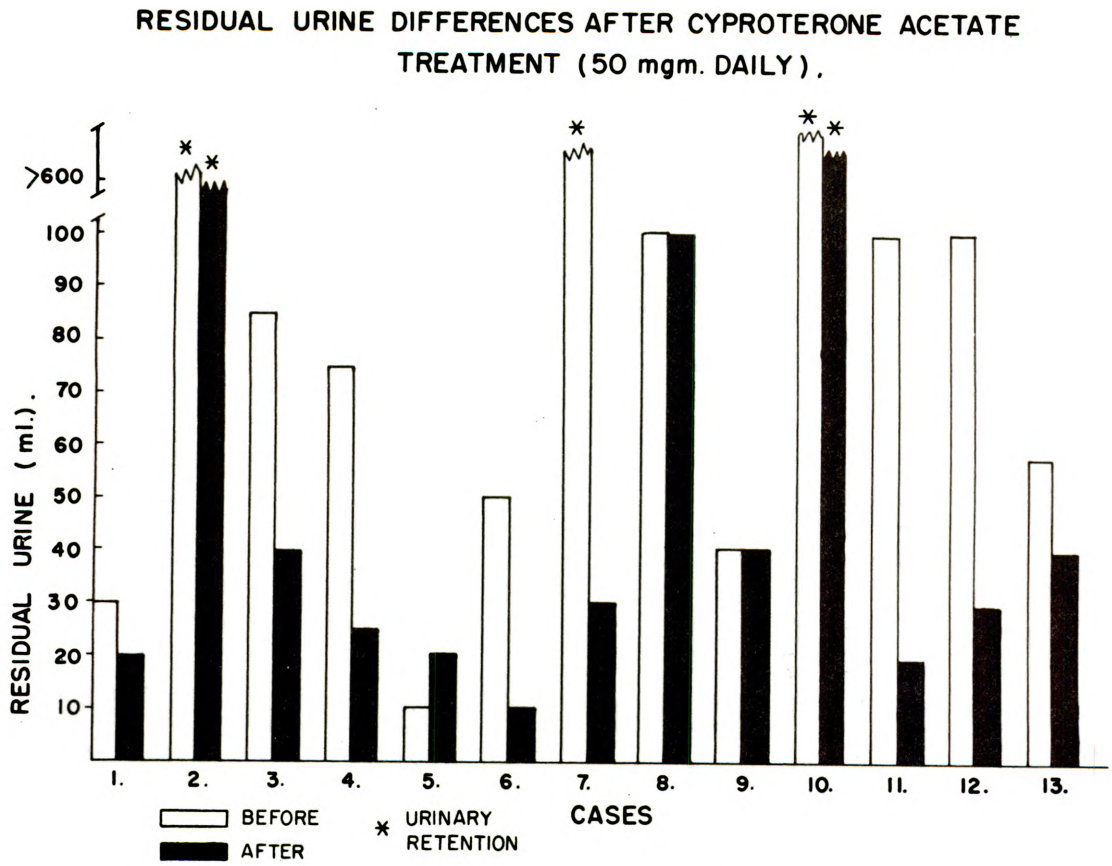

FIGURE 2. Residual urine volumes decreased in 8 of 13 patients. Patients 2 and 10 required prostatectomy. Patient 7 was in acute retention when medical treatment was begun. From Scott, W. W., and Wade, J. C. (7).

of Paulson and Kane (4). In terms of symptomatic improvement, favorable results were obtained in both series.

In an effort to answer the criticisms of some pathologists that needle biopsies are inaccurate because of the selectivity and sampling error, artifacts introduced by the needle and inadequate tissue for evaluation at times, Geller and his associates (6) performed suprapubic prostatectomy in three patients who had received hydroxyprogesterone caproate for two months. Hence, the entire specimens were available for histologic comparison with corresponding specimens from untreated patients *but* not with biopsy material from the same patient before treatment. Fig. 6 is reproduced from their study and is intended to convey a reduction in acinar size, less "inter-acinar papillation" and a change from columnar to cuboidal epithelium following two months of treatment with hydroxyprogesterone caproate.

As stated earlier, many steroids with different hormonal actions have been tried in the treatment of benign prostatic hyperplasia, and it is not the purpose of this discussion to review all these trials. The most recent double-blind, controlled clinical trial of spironolactone by Castro et al (8) showed this to be better than a placebo for the short-term treatment of benign prostatic hyperplasia, but this advantage was not maintained over a longer period of time. There was a considerable response to placebo. These authors did not present any data on the histology of these glands before and after treatment. In an uncontrolled study, Kaufman and Goodwin (9) have reported on the results of combined androgen-estrogen treatment in 42 patients with benign prostatic hyperplasia; each patient received a mixture of 25 mg testosterone propionate and 2.5 mg diethylstilbestrol intramuscularly 3 times weekly for 6 months. They reported an appreciable amelioration of symptoms in the majority of patients, improvement of voiding velocity in most, reduction of residual urine in a few, histological changes in about one-half, and some apparent reduction in size of the gland, determined by palpa-

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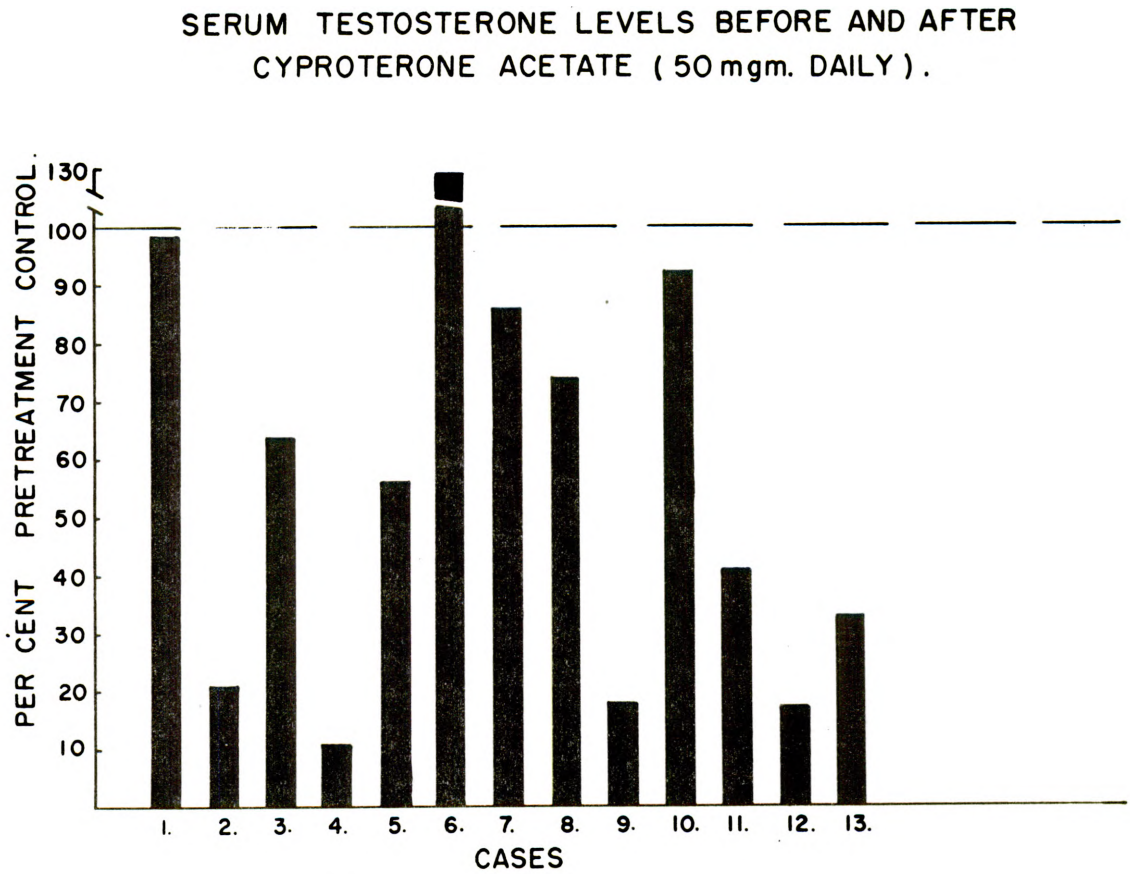

FIGURE 3. All but 1 patient showed slight to marked reduction in serum testosterone on treatment. Values are expressed in percentage of pre-treatment values, e.g., patient 4 showed almost 90 percent reduction. From Scott, W. W., and Wade, J. C. (7).

tion, in about one-third. They concluded that "these features were too inconstant and unpredictable to warrant absolute conclusions".

Most efforts to control prostatic enlargement by the administration of drugs, usually hormonally-active steroids, have in the past been based for the most part on the belief that castration results in symptomatic improvement and reduction in prostatic size in such patients and that such changes are the result of removing the major source of androgens. The role of testicular estrogens has not been emphasized as much. Before presenting our concluding remarks, it may prove useful to briefly examine a few reports on the effect of castration which have been quoted frequently in the past.

In 1895, J. William White, Professor of Clinical Surgery at the University of Pennsylvania, was first to report results in "benign hypertrophy of the prostate" after deliberate castration, claiming rapid atrophy of the prostatic enlargement in 87.2 percent of 111 cases (11). In his report, White describes the course of three of his patients in detail. He observed marked reduction in the palpable size of the prostate glands of all, this in a matter of 5 days, 12 days, and 4 weeks, respectively. He reproduced the description of the microscopic findings of a fourth patient who died 18 days following castration for prostatic enlargement. This description by Mr. Joseph Griffiths, F.R.C.S., Hunterian Professor in the Royal College of Surgeons, England, is described in detail and figures in a separate article as well (13). "In short, the cell elements first proliferate, and ultimately disappear, leaving a comparatively small amount of fibrous connective tissue in their place. . . . The gland, whether enlarged or normal, undergoes certain degenerative changes after removal of the testicles which lead to its conversion into a small, tough fibrous mass in which there are only remains of the glandular tubules and ducts."

In 1896, Doctor A. T. Cabot, surgeon to the Massachusetts General Hospital in Boston, presented a much longer follow-up on 61 of the 111 patients reported by White, noting "83.6 percent substantial

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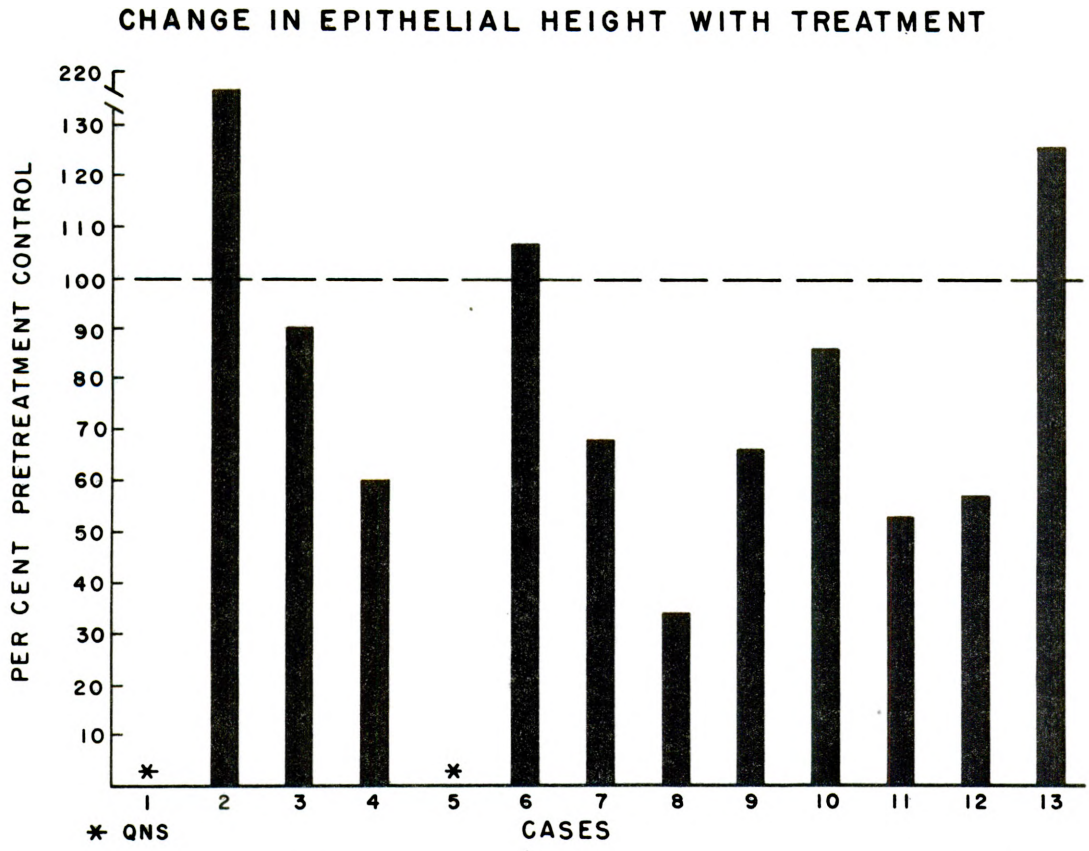

FIGURE 4. Acinar epithelial height in microns was measured without knowledge of the patient's identity or clinical states. Insufficient tissue prevented assessment in 2 patients. Decrease in height was noted in 8 of 11 patients. From Scott, W. W., and Wade, J. C. (7).

or very great improvement" (12). In his report, Cabot takes issue with the interpretations of Griffiths as follows: "The changes thus described are so closely similar to those which he thinks he has found in a case of castration, examined after eighteen days, that there might well be doubt whether the condition which he thought due to the effect of castration might not have been one of degeneration already started before castration was done".

These reports were quickly followed by other publications of failure of relief of prostatic enlargement following castration, and some 30 to 40 years elapsed before Deming and his associates at Yale revived the subject (14,15). In their first paper, they report no effect of castration on prostatic enlargement in one patient who was castrated initially and whose failure to improve necessitated open prostatectomy about one month later; in their second paper, they present a second patient in their argument against castration. This patient, age 74, had one cm testes, probably as the result of injury to each separately at ages 18 and 21 years. At open operation, a sizeable median and two lateral lobes were removed, as well as one testis. Fig. 7 shows the gross specimens. Fig. 8 is a photomicrograph of the prostate removed, showing the usual nodular hyperplasia, and Fig. 9 is a photomicrograph of the so-called "atrophic" testis, showing sheets of interstitial cells. The authors suggested that in this patient benign prostatic enlargement developed in the presence of testicular atrophy, but it is quite likely that these small testes with abundant interstitial cells were functional from a hormonal standpoint. Certainly, this man was not a castrate, as he has been quoted as being.

In 1940, Huggins and Stevens (16) re-examined the problem, believing that determination of the effect of castration on benign hyperplasia is one of the most critical ways to settle the question of whether such growths are under endocrine control. Three patients were studied. In case 1, no change was noted in the number of acini, their diameter, nor the height of the epithelial cells in a period of 29

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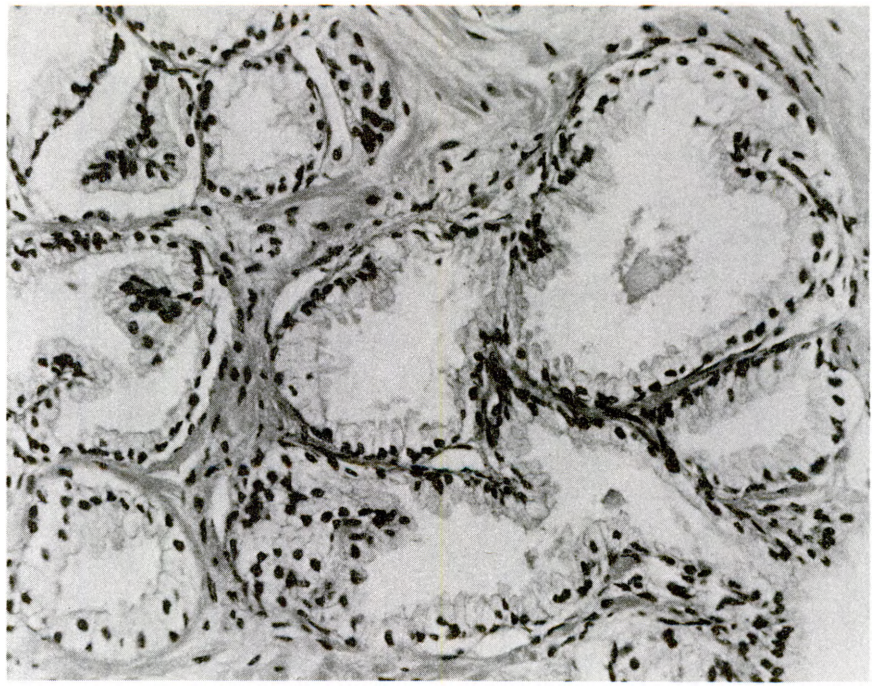

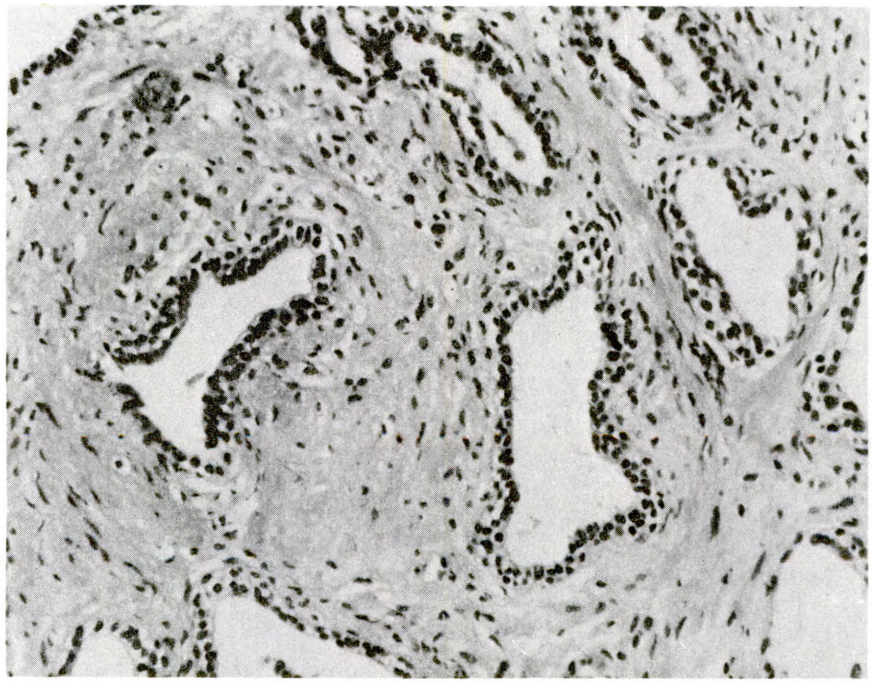

FIGURE 5. Patient 8. Histological appearance of prostatic tissue obtained by needle biopsy before (A) and after (B) treatment with cyproterone acetate. H and E x275. From Scott, W. W., and Wade, J.C. (7).

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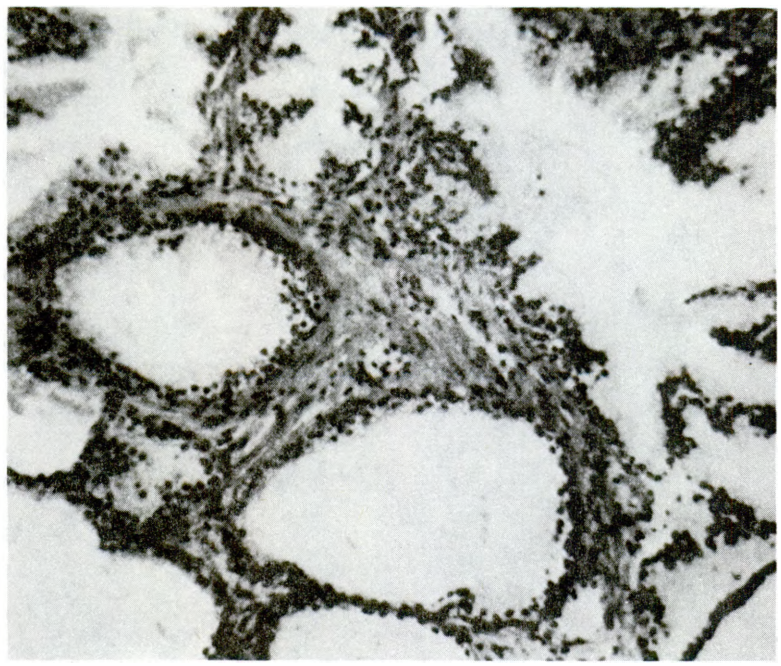

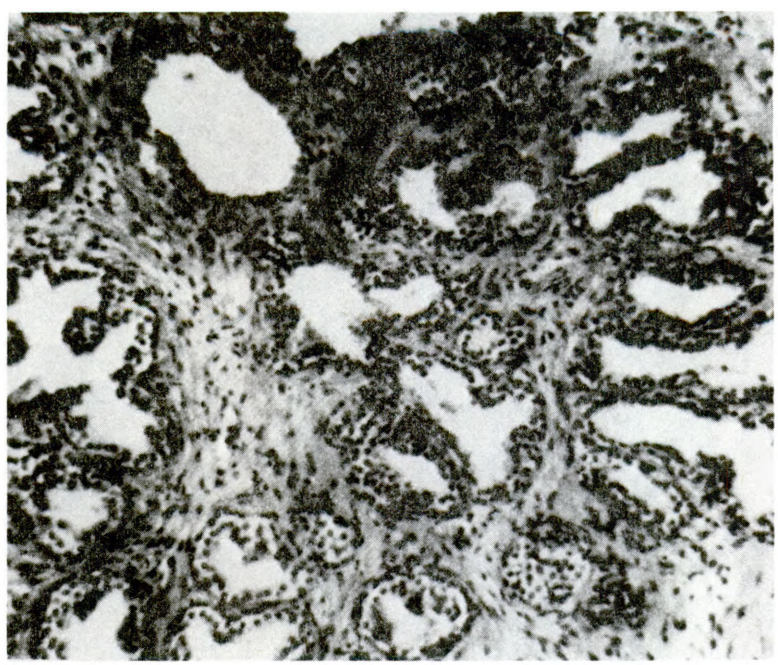

FIGURE 6. (A). Appearance of untreated human benign prostatic hyperplasia at intermediate zone in nodule. Acini are medium in size and lined by simple, tall, columnar epithelium. Note intra-acinar papillation. (B). Similar cells treated with hydroxyprogesterone show small acini lined by cuboidal epithelium with subapical or midzonal nuclei. Basal cells prominent in place. H. and E, slightly reduced from x150. From Geller *et al.* (6).

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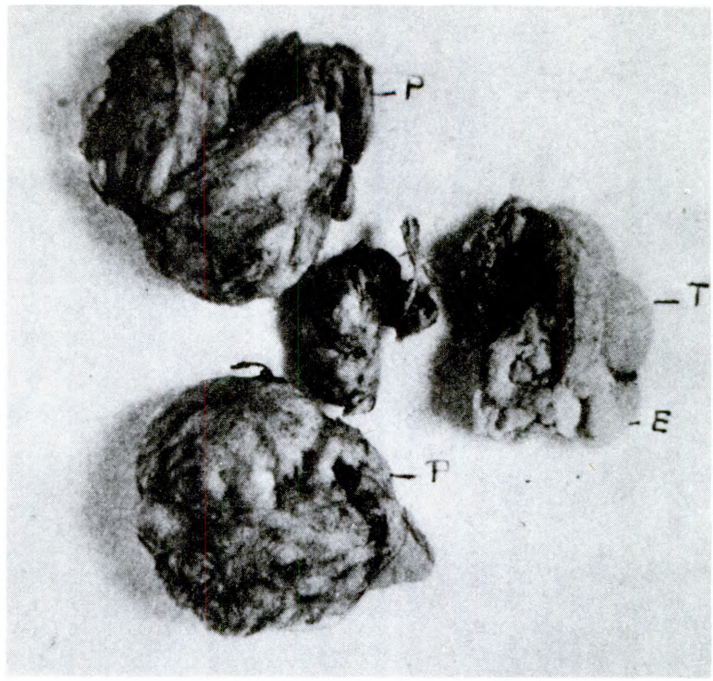

FIGURE 7. Photograph. P, prostatic tissue remove as recorded in case report; T, testicle; E, epididymis. (From Deming *et al.* (15).

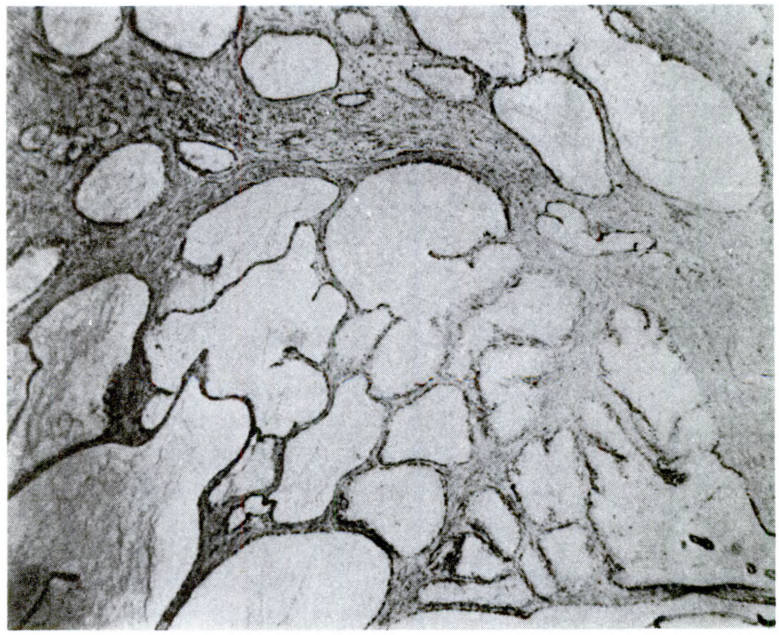

FIGURE 8. Photomicrograph (x75) of prostatic adenomia in case reported. From Deming *et al.*, (15).

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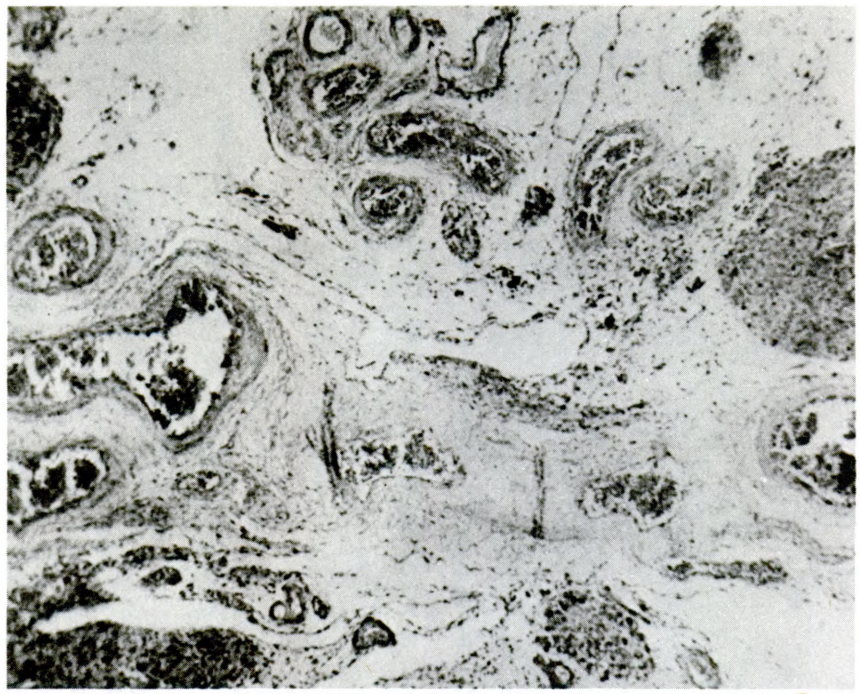

FIGURE 9. Photomicrograph (x75) of testis showing sheets of large mononuclear cells resembling interstitial cells which are separated by loose vascular connective tissue and some adipose tissue. No tubules or their remains can be made out. From Deming *et al.* (15).

days following castration (Figs. 10A and 10B). In case 2, histological examination of prostatic tissue obtained 86 days after castration revealed an increase in the number of acini, these acini being smaller and lined with cuboidal epithelium, when compared with prostatic tissue obtained before castration (Table I, Figs. 11A and 11B). This patient also had disseminated prostatic cancer. In case 3, no histological sections are reproduced, but were described: "Section of prostate 91 days after castration: Most of the tissue consists of fibromuscular stroma with relatively infrequent small acini. The acinous epithelium is cuboidal and is stratified in layers of two and three cells. The epithelium resembles the postorchietomy specimen in case 2." Huggins and Stevens concluded that: "Epithelial atrophy was not present 29 days after castration but appeared plainly 86 and 91 days after the operation. (and) The evidence derived from castration on benign prostatic hypertrophy in man supports the view that the prostatic epithelium at least is under control of the testes".

Believing that more data are necessary to draw conclusions between established benign prostatic hyperplasia and the testes, and recognizing that purposeful castration of a large number of men with this condition is not feasible, Wendel, Brannen, Putong and Grayhack in 1972 (17) compared the histology of benign hyperplasia in patients with prostatic carcinoma who had been treated by castration and estrogen therapy with the histology of benign hyperplasia in patients with prostatic cancer who were not treated. Their conclusions were: "The benign prostatic hyperplasia present in patients with carcinoma of the prostate treated with orchiectomy and estrogens is of a lower grade than in those patients not having this treatment. Infolding of the acinar epithelium and the secretory phase are virtually absent in the group having orchiectomy and estrogens (Fig. 12A), as compared to their presence in patients not having this treatment (Fig. 12B). The findings in patients with occult or untreated prostatic cancer suggest that the differences are correlated with orchiectomy and estrogens and not with the presence of carcinoma itself. These observations support the concept that cells of benign prostatic hyperplasia are subjected to continuing stimulation by the testes."

Studies such as this are helpful in our approaches to hormonal regulation of benign prostatic growth, but many questions remain unanswered. Thus, the role of testicular estrogens in such growth is not as yet resolved, nor is the effect of castration on the fibromuscular stroma clear.

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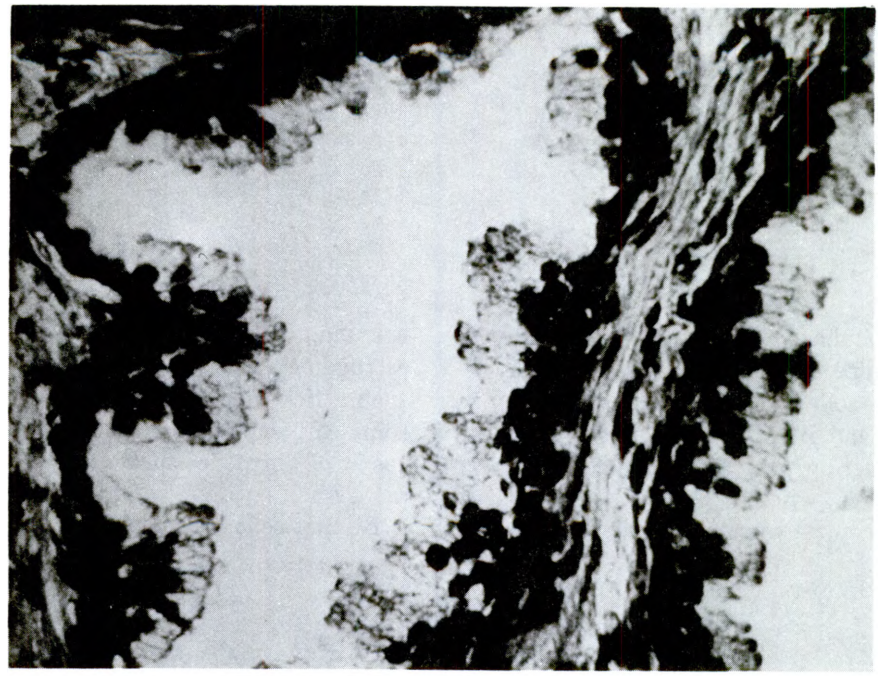

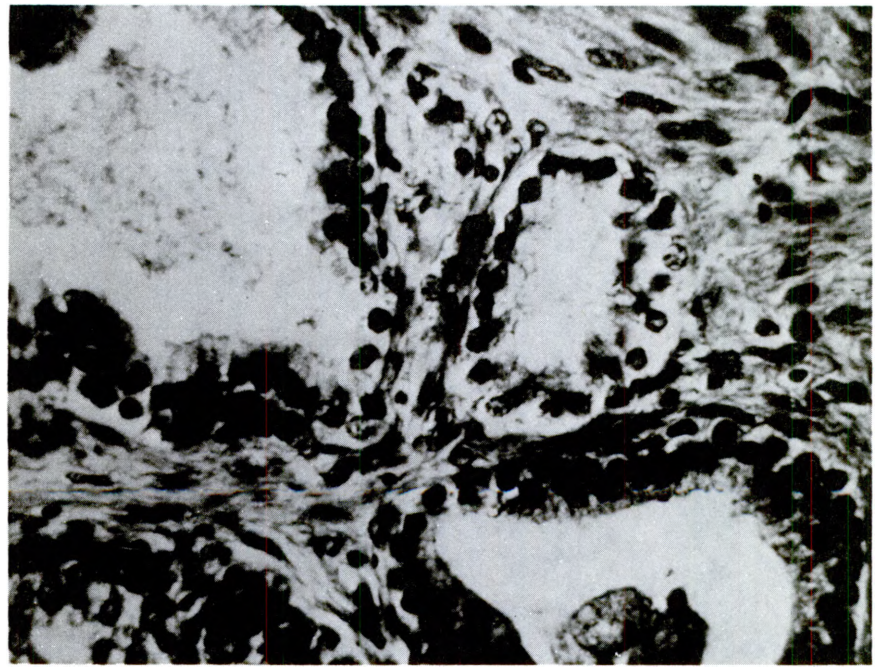

FIGURE 10. A, Benign hypertrophy of prostate before castration (x550); B, Section of adenomatous prostate from same patient 29 days after castration (x550). From Huggins, C., and Stevens, R. A. (16).

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TABLE I—Measurements of epithelium in prostatic hypertrophy in 50 noncastrated cases, as well as before and after castration in 3 men

	NUMBER ACINI PER MICROSCOPIC FIELD (100 ×)	DIAMETER OF ACINI	HEIGHT OF EPITHELIAL CELLS
		mm.	*micra*
Average measurements in 50 noncastrated cases with probable error	24.9 ± 10.4	0.46 ± 0.12	14.4 ± 2.3
Case 1, before castration	32	0.38	11.8
Case 1, 29 days after castration	29	0.33	11.8
Case 2, before castration	19	0.64	14
Case 2, 86 days after castration	34	0.20	8
Case 3, 91 days after castration	18.4	0.24	8.8

In leaving this phase, if you are not already confused, you perhaps will be, as I was, when I saw and reported the following sections some years ago (18). Figs. 13A and B represent sections from the enlarged, obstructing prostate removed surgically from a man, age 60, who had been castrated at age 28. One shows typical hyperplasia and one classic squamous metaplasia. If these are androgenic and estrogenic effects, respectively, as some believe, such action must have occurred prior to castration some 32 years before, developed afterwards as a result of hormones formed extragonadally, or hormones had nothing to do with it.

Be that as it may, most of us working in this area

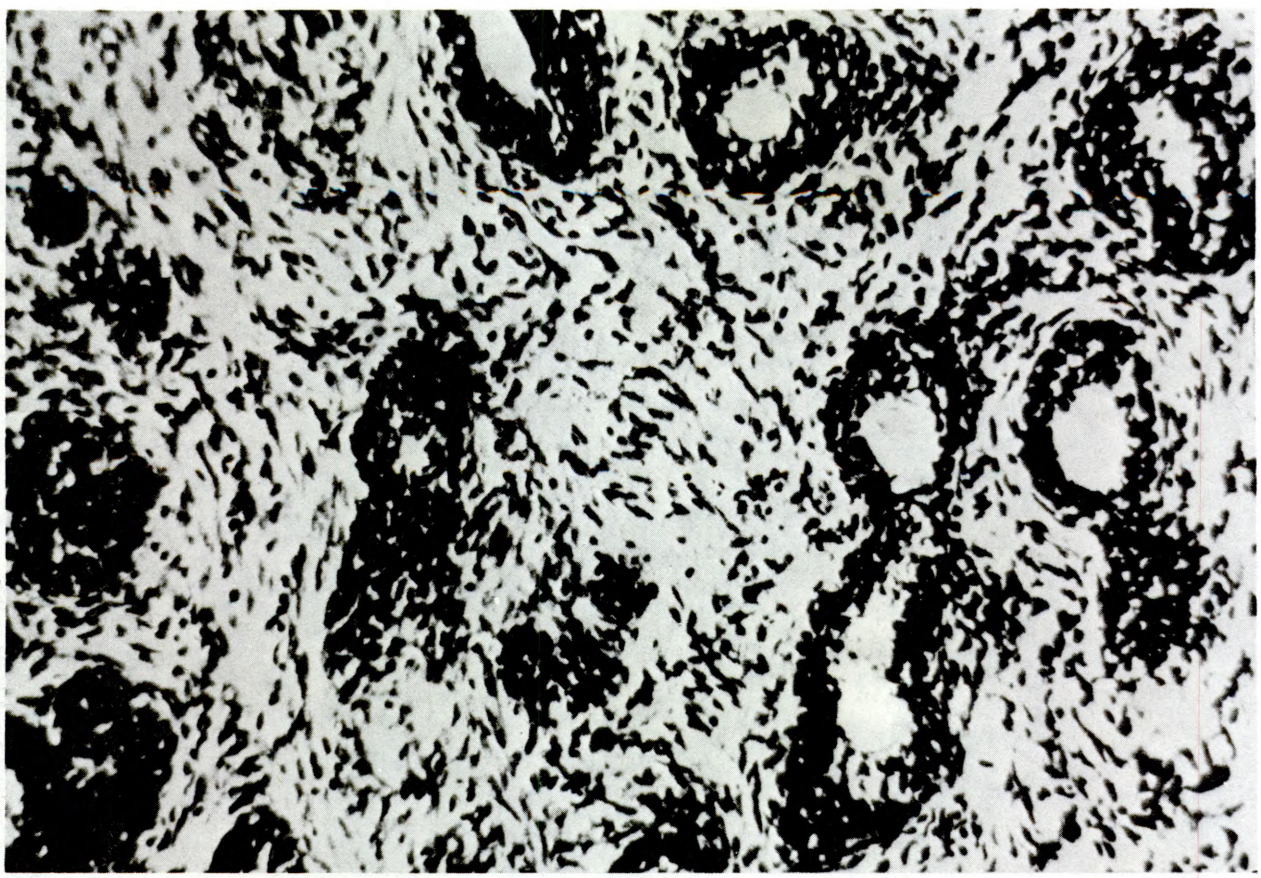

FIGURE 11. Case 2. A, Benign hypertrophy of prostate before castration (x235); B, Section of prostate 86 days after castration.

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believe that the problem is soluble. With the possible exception of the dog, we lack an animal model of BPH and consequently must proceed with studies in humans. We must be forever mindful that the functional results presently achieved by surgical removal are good. Medical management, to be acceptable, must offer results which are just as good and are free from untoward side effects.

Summary

Evidence suggests that castration in man is followed by distinct retrogressive changes in the epithelial component of the benign nodular hyperplastic prostate, the changes occurring in the fibromuscular portion being less clear. Similar changes have also been observed in man following the administration of several progestational agents. Such observations suggest a search for inhibitors of the fibromuscular components, as well as the role of testicular estrogens in such growth.

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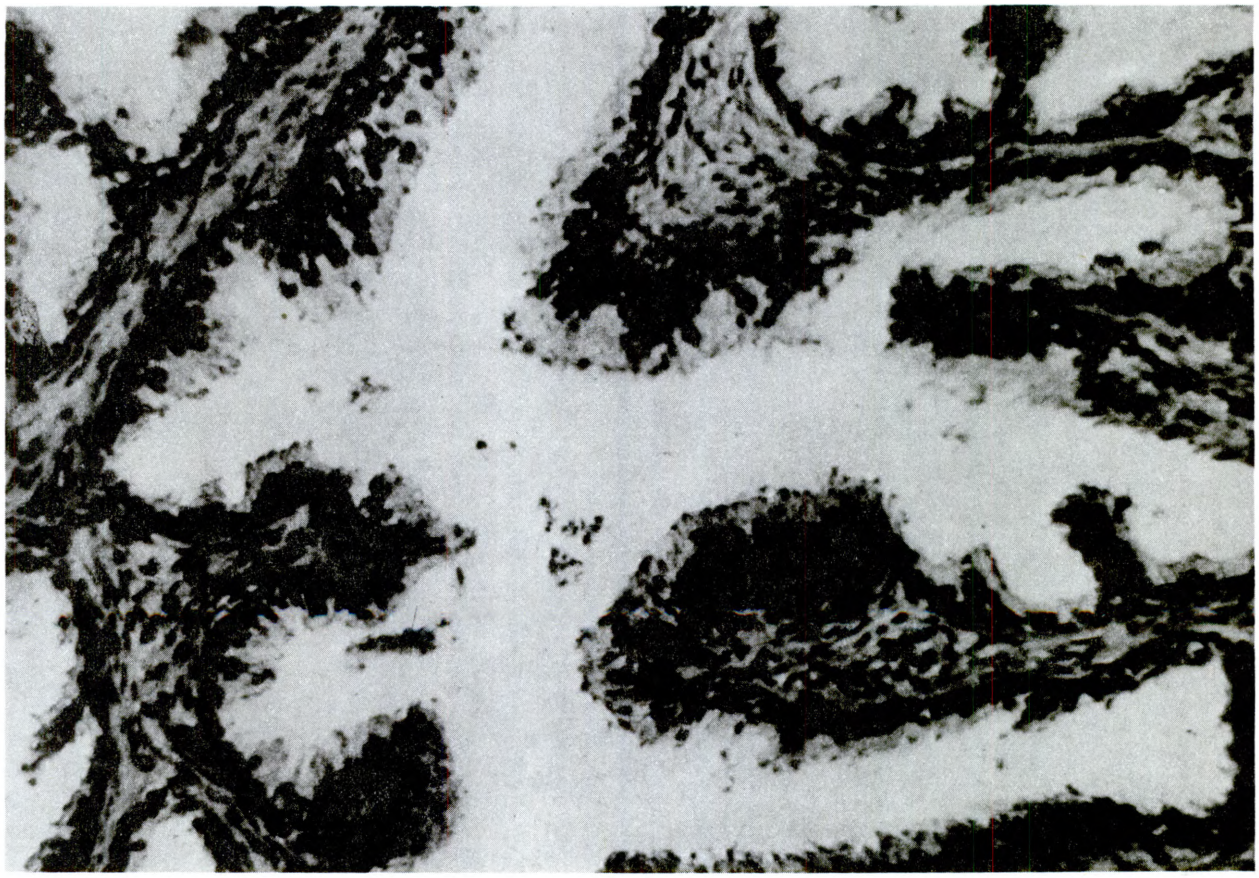

FIGURE 11 (cont.) This section showing epithelial atrophy is typical of most of the gland (x235). From Huggins, C., and Stevens, R. A. (16).

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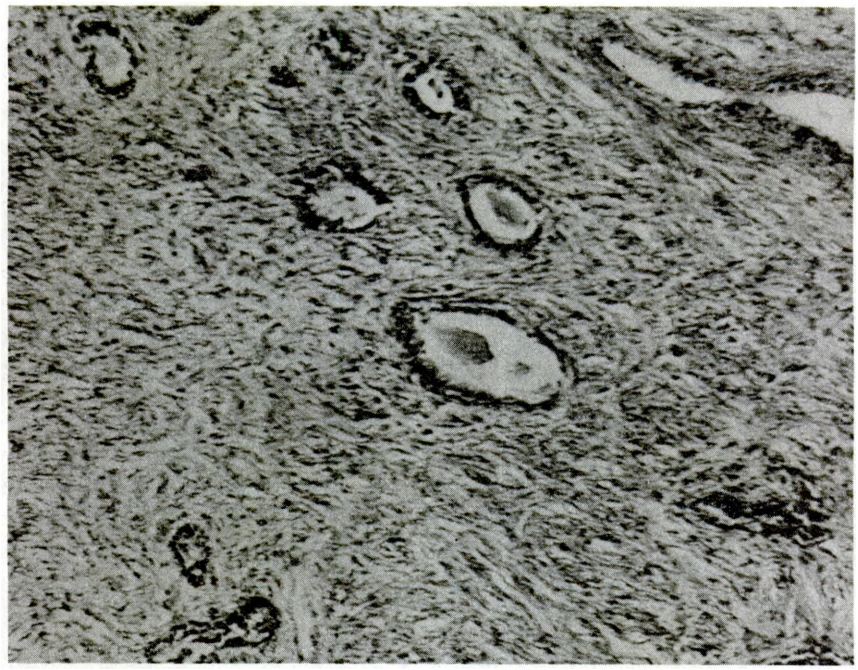

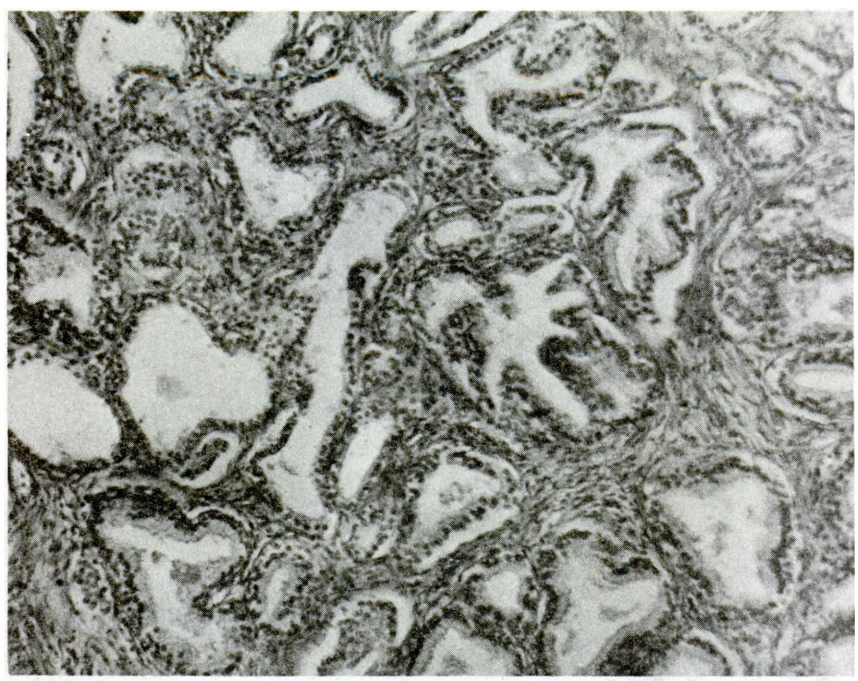

FIGURE 12. A, Photomicrograph of prostatic tissue in patient treated with orchiectomy and estrogen; B, Photomicrograph of prostatic tissue in patient with untreated prostatic cancer. (Both reduced from x100) From Wendel, E. F., *et al.* (17).

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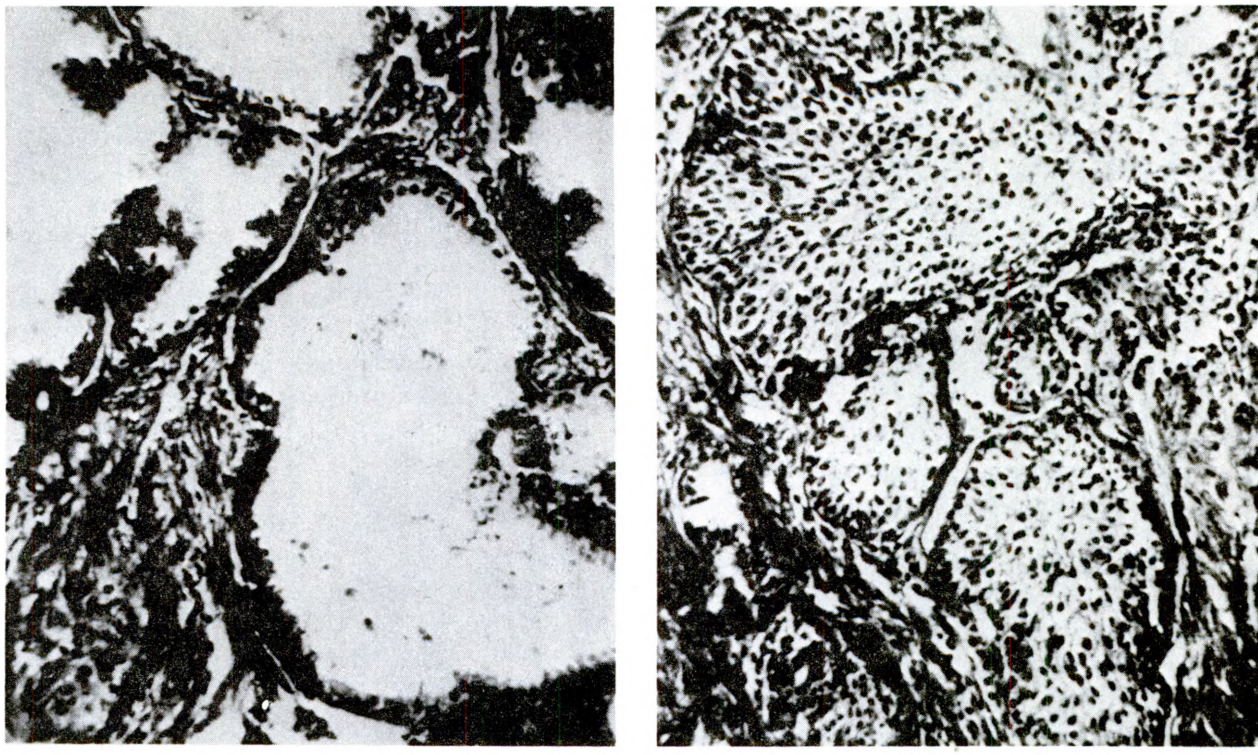

FIGURE 13. Sections from prostate gland of a man, aged 60 years, whose testes were removed at age of 28 years because of tuberculous epididymitis and orchitis. A shows typical benign prostatic hyperplasia; B shows classic squamous metaplasia. Both sections x150. From Scott, William W. (18).

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DISCUSSION

R. HORTON: Many of the concepts that are being

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discussed in this workshop have to do with testosterone uptake and metabolism in the prostate cell. This may be the time to encourage studies in which synthetic steroid androgens are evaluated in terms of their effect on LH and testosterone levels in patients with BPH rather than the use of substances such as antiandrogens that interfere with androgen action in male accessory tissues. And, it would seem to me that there are some synthetic androgens that may not be potentially converted into reduced A-ring steroids such as dihydrotestosterone.

J. D. WILSON: You are suggesting that LH could be inhibited by some steroid that does not have a growth promoting effect on the prostate?

HORTON: Yes. I'd like to see some tests to evaluate synthetic androgen, particularly if they could not be converted into some of the post-testosterone compounds that we are hearing about in this Workshop. That would provide a circulating androgen which by not being converted into final messengers for testosterone action in prostate might be effective clinically as well as biochemically.

WILSON: All the androgens I can think of either themselves bind actively once they are effective androgens, or they circumvent the need for reduction such as the series that Dr. Liao studies, the 19-Nor steroids.

HORTON: What I am hoping for is a pure anabolic agent, perhaps not even a steroid. It would produce anabolic effects, but it would also produce selective deandrogenization of the prostate.

D. F. PAULSON: One of the problems in the manipulation of the adult elderly male is that very few people are willing to trade prostatism for impotence. Many of the drugs that are currently on trial do result in impotence to the dissatisfaction of the prospective patient. On the clinical side, Dr. Scott gave an excellent presentation about the problems in establishing objective parameters of response to treatment in this population group. Most males in a study will respond to a certain degree and not necessarily from the drug.

J. T. GRAYHACK: I tend to sympathize with your suggestion; if the testis makes a hormone other than androgen that is a factor in this pathologic process, you could probably avoid the risk of impotence by giving an androgen to shut off the individual's own testes. It has never been demonstrated clearly that giving androgen *per se* stimulates BPH. We had androgen as one drug in a triple blind study and could see no real effect on the histology of BPH. I don't think that anybody's ever done what you suggested in the way of controlling the level administered.

S. G. KORENMAN: I think there is evidence for agents that are more anabolic than androgenic, and it is conceivable that an androgen-estrogen combination with a slightly diminished total androgenic effect but that caused inhibition of gonadotrophins could be effective in the right proportion.

C. DESJARDINS: I don't know why people believe that estrogen is a better inhibitor of LH secretion than testosterone *per se*. We have conducted some experiments in which we suppressed the testes with estrodiol or testosterone implants and found that circulating levels of LH and FSH were equivalent in both groups.

H. BRENDLER: About 20 years ago there were a number of studies on combination estrogen-androgen therapy in BPH as you doubtless know, and one of them by J. J. Kaufman and W. E. Goodwin reported beneficial results.

J. GELLER: Regarding Dr. Horton's suggestion that BPH be controlled with a synthetic androgen that would not be metabolized to a 5α-reduced androgen, I'm not certain that this would be effective since circulating adrenal androgens can still be converted to DHT within the prostate. As Dr. Scott pointed out, if the hypothesis that BPH is due to a testicular hormone has any validity, we are now in a position to test the hypothesis with antiandrogens that decrease circulating testosterone levels, block androgen receptor, and decrease dihydrotestosterone formation in the prostate. Such biochemical changes could then be correlated with a clinical parameter of prostate obstruction such as the pressure-flow studies mentioned by Dr. Paulson. Following patients of this type to surgery and measuring the histological and biochemical parameters of the prostate in correlation with the clinical changes might allow us to determine whether there is any merit in the concept of androgen causation of BPH.

L. M. FRANKS: I think you are asking for something that is difficult, if not impossible, because you are asking for an antiandrogen that only inhibits certain androgenic effects. What you are looking for is something that blocks locally just in the areas that are producing obstruction and does not have any effect on the areas that control sex drive. Perhaps the logical answer is to look for a blocking agent that is organ specific.

O. HECHTER: The notion that we may develop an antiandrogen that would selectively inhibit androgenic effects in prostate, but that would not effect androgenic activities in other tissues and or-

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gans, reminds me of discussions we used to hear many years ago in the steroid field. The thing then was to develop a glucocorticoid analog by appropriate molecular "tailoring" that would inhibit inflammation but would not exhibit other glucocorticoid activities; thus the problem of "side effects" would be eliminated. There was also a search for an "anabolic" steroid, a synthetic androgen analog that would act exclusively on muscle mass and not exhibit androgenic effects on other tissues. Estrogen analogs that act to reduce the level of blood lipids but that would not exhibit classical estrogen activities were also sought. This structure-function approach yielded a large series of interesting steroid analogs. The ratio of biological activities with some analogs were indeed modified somewhat toward the desired end. However, the limitations of this approach were soon realized. The best anti-inflammatory steroid retained classical glucocorticoid activities; anabolic steroids retained androgenic activity, and hypolipemic estrogen analogs retained estrogenic activities. Given this past history, I wonder why this type of idea is now being resurrected.

FRANKS: This, I think, is the whole point. The question is the right question but the answer is not necessarily to look at it from a systemic endocrine point of view.

WILSON: I don't agree with either of your remarks. Among all steroid hormones, androgens are unique in the sense that what circulates in blood is not the physiologically active steroid in many instances. There is a large body of evidence for example, that sex drive is caused by a combination of androgen metabolites, that androgen-mediated growth is caused by others and that in some cells testosterone itself is an active hormone. This is one situation in which what was considered possible on theoretical grounds several years ago is not inconceivable.

HORTON: And that is what led to my comment, that peculiar situation in regard to androgen metabolites.

WILSON: Most physiological effects of testosterone are cumulative effects of the estrogenic and androgenic metabolites. Some purely androgenic effects are produced by dihydrotestosterone which cannot serve as a substrate for estrogen formation. Others are served by testosterone alone and some are produced by combinations of metabolites. We are just beginning to understand this complex interrelation.

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HYPOTHALAMUS-PITUITARY FUNCTION: LH CHANGES WITH AGE

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Recent use of sensitive and specific radioimmunoassays for luteinizing hormone (LH) and plasma sampling at frequent intervals have led to important advances in our understanding of the ontogeny of LH secretion. The importance of sleep in the secretion of human growth hormone (1–3), prolactin (4,5) and pubertal LH (6) has been demonstrated. The finding of augmented LH secretion synchronous with and occurring only during sleep in early pubertal children showed the importance of obtaining samples at frequent intervals for the complete 24-hour sleep-wake cycle. Late pubertal subjects showing advancing sexual maturation also exhibit episodic LH secretion during waking. After the completion of sexual maturation, there is no consistent difference in the mean LH concentration during sleep compared with waking (7).

Before one can make an assessment of the relationship of a hormone to a specific disease, such as benign prostatic hyperplasia (BPH), one must measure the hormone in the appropriate biologic fluid over an appropriate time period, as well as study a control population matched for age, sex and "non-specific effects of illness" such as nutritional status, medication and coexistant disorders. In an attempt to provide a sound foundation for studying changes in LH secretion in patients with BPH, we will attempt to review the ontogeny of LH secretion from fetal life until old age.

Materials and Methods

In the studies that were performed in our laboratory, plasma LH was measured by the double antibody radioimmunoassay utilizing reagents supplied by the NIAMDD (8). The results are expressed in terms of the 2nd International Reference Preparation-Human Menopausal Gonadotropin (2nd IRP–HMG). All samples from each subject's 24-hour study were assayed simultaneously to negate interassy variability. During nocturnal sleep, polygraphic monitoring was carried out to define sleep onset, wakefulness and specific sleep stages that were scored according to standardized criteria (9). Student's t test was used to determine the significance between the mean LH concentration asleep compared to waking. Pubertal stage was assigned according to the criteria of Tanner (10). Frequent interval plasma sampling studies were performed as previously reported from this laboratory (11). The pubertal subjects were between 10 and 16 years of age, and the adult men were between 22 and 45 years of age.

Results and Discussion

Fetus: The presence of pituitary cells that show the characteristics of gonadotropes have been identified as early as the eighth week of gestation (12). Grumbach and Kaplan (13) found that six of eight fetuses had measurable pituitary LH (1.8 to 105 ng) as early as 68-80 days gestation. The mean pituitary LH content in the fetus increased from 82 ng to 1906 ng at 25–29 weeks, a level twice that of new-

Supported by a grant (RR–53) from the General Clinical Research Centers Branch of the National Institutes of Health and by research grants (CA 07304 and HL–1473F) from the U.S. Public Health Service.

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borns. Serum "LH–HCG" [1] was dectable at 84 days gestation (14) and increased to its maximum level between 84 and 140 days when it began to fall ultimately to below 10 ng/ml at term.

First Year of Life: Plasma immunoassayable LH levels are high during the first year of life. Forest et al (15) reported a mean LH level of 19.7 mU/ml for male infants one to twelve months of age. These elevated levels decreased to 3.9 mU/ml after the first year and remained at this low level until the onset of puberty. Buckler and Clayton (16) showed that urinary LH was also increased during the first 6 months of life. These combined observations suggest that LH production is high during the first year of life. Whether these elevated values result from failure of maturation of the systems concerned with metabolism of LH or are due to the presence of biologically inactive subunits remains to be elucidated.

Prepuberty: The period encompassing the beginning of the second year until the onset of episodic LH secretion during sleep can be considered the prepubertal period. Early prepubertal children show LH levels that are low and oscillate around a relatively low mean value. There is no significant augmentation of LH secretion during sleep (Fig. 1). The presence of augmented LH secretion during sleep that has been shown to stimulate testosterone secretion (17) heralds the onset of clinical puberty. Although urine LH concentrations are low in prepubertal children, Rifkind et al (18) reported the finding of increased urine LH levels every 4–5 days when daily sequential 24-hour collections were made. These findings suggest that prepuberty may differ from "late prepuberty or early puberty only in terms of the frequency with which augmented LH secretion during sleep occurs.

"Late" Prepuberty and Early Puberty: The finding of augmented LH secretion synchronous with sleep (Fig. 2) has been shown consistently in "late prepubertal and pubertal subjects (6,19). This augmented secretory activity during sleep in pubertal subjects results in consistently higher mean LH concentrations during sleep compared to waking (Table 1). The important role of sleep, rather than the time at which sleep occurs has been demonstrated by delayed sleep onset (6) and sleep reversal studies (20) where a shift in augmented LH secretory activity to the new sleep period occurs. It should be emphasized that some clinically prepubertal boys will show this pubertal LH "program" since this is what initiates testosterone secretion and the resultant development of secondary sex characteristics. The age and/or mechanism(s) that determine the time of initiation of this "program" are incompletely understood. A possible important role for adrenal androgens (21,22) as well as the attainment of a "critical" body composition (23) have been suggested.

Late Puberty: Late pubertal boys begin to show episodic secretion of LH during waking (Fig. 3) although the mean LH concentration during sleep remains significantly higher (Table I). During this pubertal stage, testosterone secretion has been initiated and begins to exert a negative feedback effect on LH secretion. After the completion of sexual maturation, mean waking LH levels are equivalent to mean LH levels during sleep. The absence of a consistent augmentation of LH secretion during sleep is characteristic of sexually mature men (7,24).

Adulthood: The pattern of LH secretion in adult men is characterized by periods of abrupt increases in the plasma LH concentration followed by slower declines (Fig. 4). The calculated half-lives from the linear log decrement of a secretory episode are longer than that measured after hypophysectomy (25). This probably results from persistent secretion of LH during the declining slope of a secretory episode. Because of the episodic nature of LH secretion in adult men it is clear that single plasma determinations must be interpreted with caution. The 24-hour mean LH concentrations in a group of normal men 22–45 years of age are shown in Table II. Recently, Santen and Bardin (26) showed that

TABLE I—Mean Plasma LH Asleep and Awake During Puberty

Subject	Pubertal Stage	Age	LH (mIU/ml)	
Early Puberty	P2, P3		Asleep*	Awake
1		12	4.9	3.4
2		15	10.1	4.3
3		10	8.9	7.3
4		15	7.4	4.1
5		13	14.4	6.1
6		14	11.0	6.3
7		12	6.2	2.5
Late Puberty	P4, P5			
8		15	11.0	8.2
9		13	12.5	8.6
10		16	16.2	13.0
11		15	6.0	2.8
12		15	12.3	7.7

*Mean LH asleep significantly greater than mean LH awake ($P<0.001$).

[1] The antibody used in these studies did not distinguish LH from HCG.

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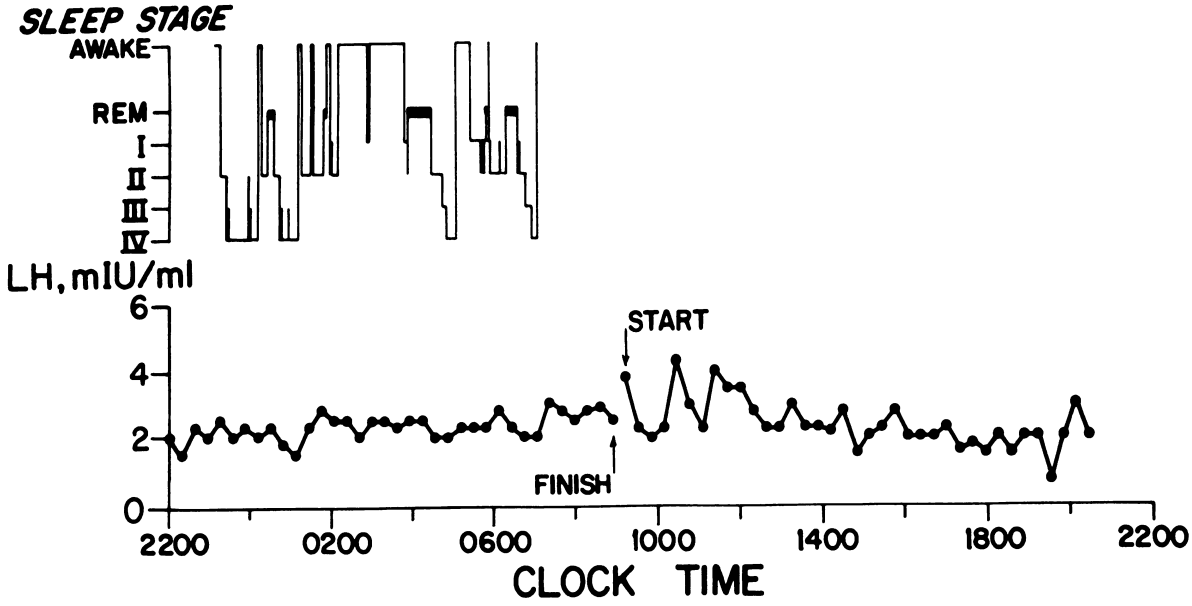

FIGURE 1. The 24-hour LH secretory pattern derived from 20-minute interval plasma sampling in an early prepubertal child. The sleep histogram is graphically depicted above the period of nocturnal sleep. Sleep stages are designated REM (rapid eye movement) = ▬, and stages I-IV by the depth of the line graph.

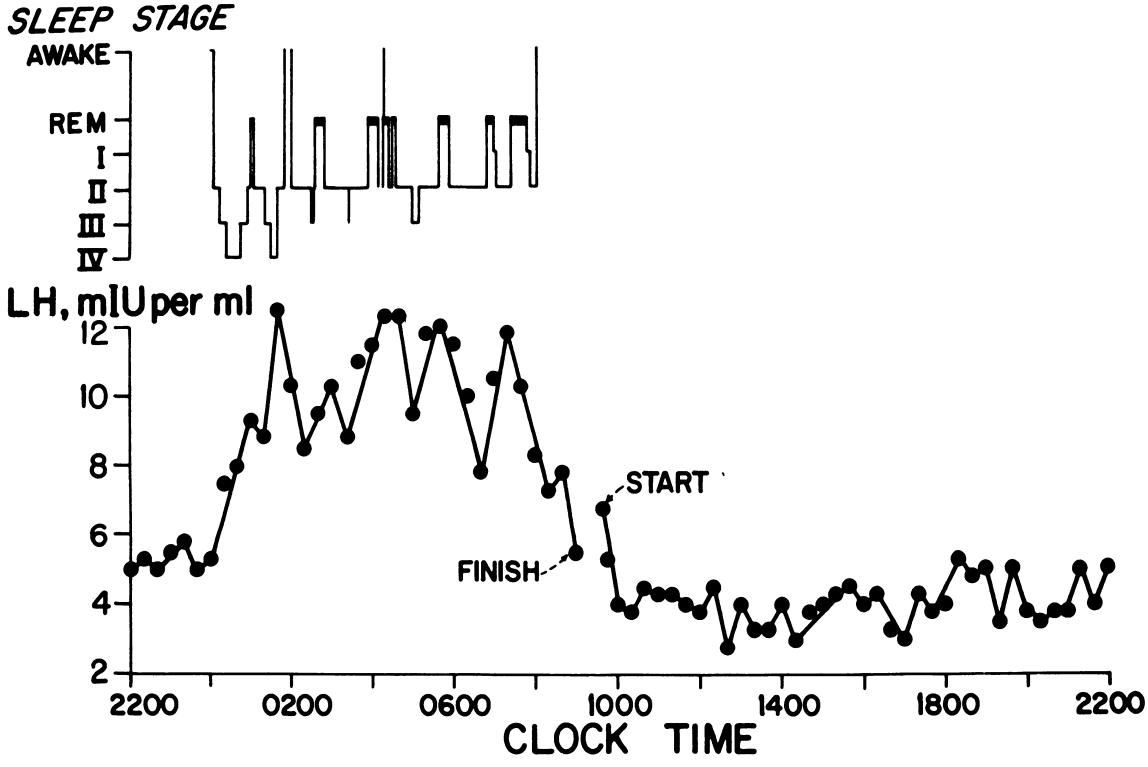

FIGURE 2. The 24-hour LH secretory pattern and sleep histogram in a normal early pubertal boy. Note, the augmented LH secretory activity during sleep.

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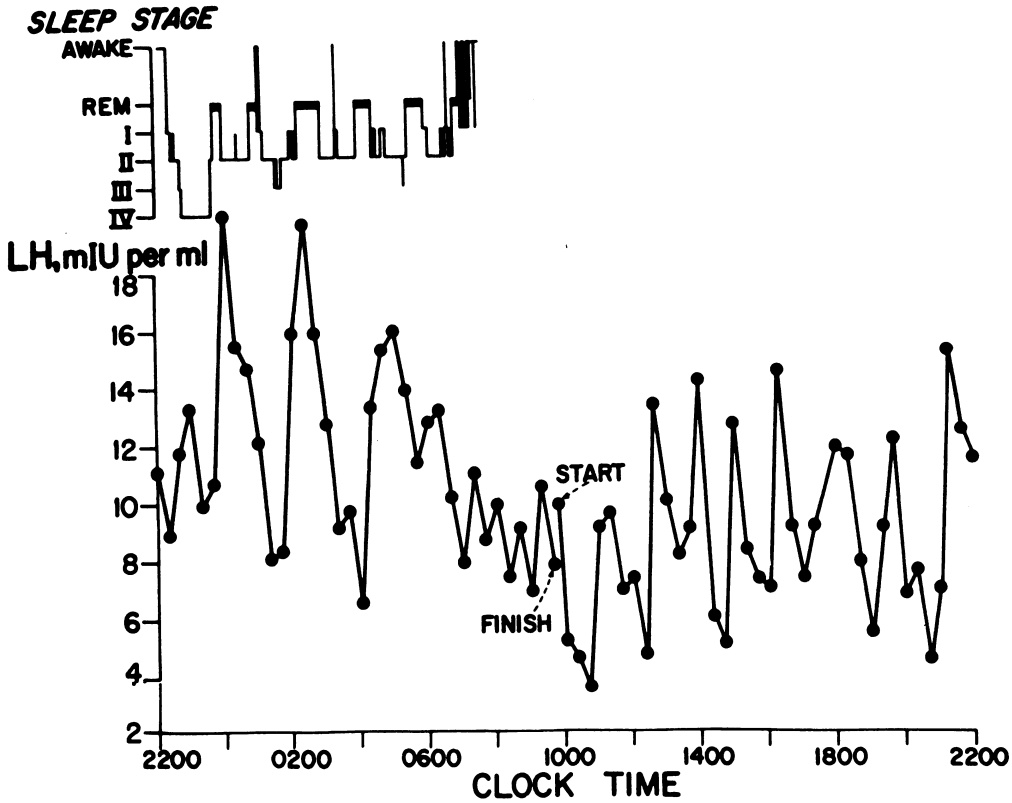

FIGURE 3. The 24-hour LH pattern and sleep histogram in a normal late pubertal boy (Tanner Stage P4). Note, the episodic secretion of LH during waking.

the mean LH concentration calculated from 20 minute interval plasma samples over 3 hours gives an accurate mean value compared with the cumulative mean obtained by 6 hours of 20-minute interval sampling. This 3 hour sampling period seems to be a reasonable compromise in minimizing the problem of episodic LH secretion. Since there is no significant effect of sleep in adult men, plasma sampling during this time period can probably be eliminated.

LH Changes with Aging (Table III): There are relatively few reports of plasma LH changes during aging. Stearns et al (27) found increased LH levels in 54 of 86 (62%) men above the age of 70. Nieschlag et al (28) reported a mean LH level of 13.1 mU/ml in men 60–90 years of age compared to 9.0 in men 20–60 ($P < 0.001$). Rubens et al (29) found a mean LH value of 13.0 mIU/ml in men less than 50 compared to 24.1 mIU/ml in men above 65 ($P < 0.001$). Recently, Isurugi et al (30) reported significantly higher ($P < 0.01$) LH levels in men during each decade after 41 compared to a group of men 16–40 years of age. All these studies suggest that plasma LH increase significantly after age 50. Since these data were obtained from "single" plasma levels, they must be considered tentative until more fre-

TABLE II. 24-Hour Mean Plasma LH in Normal Adult Men

Subjects	Age	24-Hour Mean LH (mIU/ml)
1	40	7.4
2	38	5.5
3	24	11.8
4	22	7.4
5	23	3.7
6	22	7.0
7	45	20.7
8	45	6.7
9	45	8.1
Mean	34	8.7

TABLE III. Plasma LH Levels in Men During Aging

Authors	Age (Yr)	LH	Significance
Stearns et al. (1973)	>70	54/86	
Nieschlag et al. (1973)	20–60	9.0 mU/ml	P<0.001
	60–90	13.1	
Isurugi et al. (1974)	16–40	22.3 ng/ml	
	41–50	32.5	P<0.01
	51–60	57.4	P<0.01
	61–70	69.8	P<0.01
	70	60.9	P<0.01
Rubens et al. (1974)	50	13.0 mIU/ml	P<0.001
	65	24.1	

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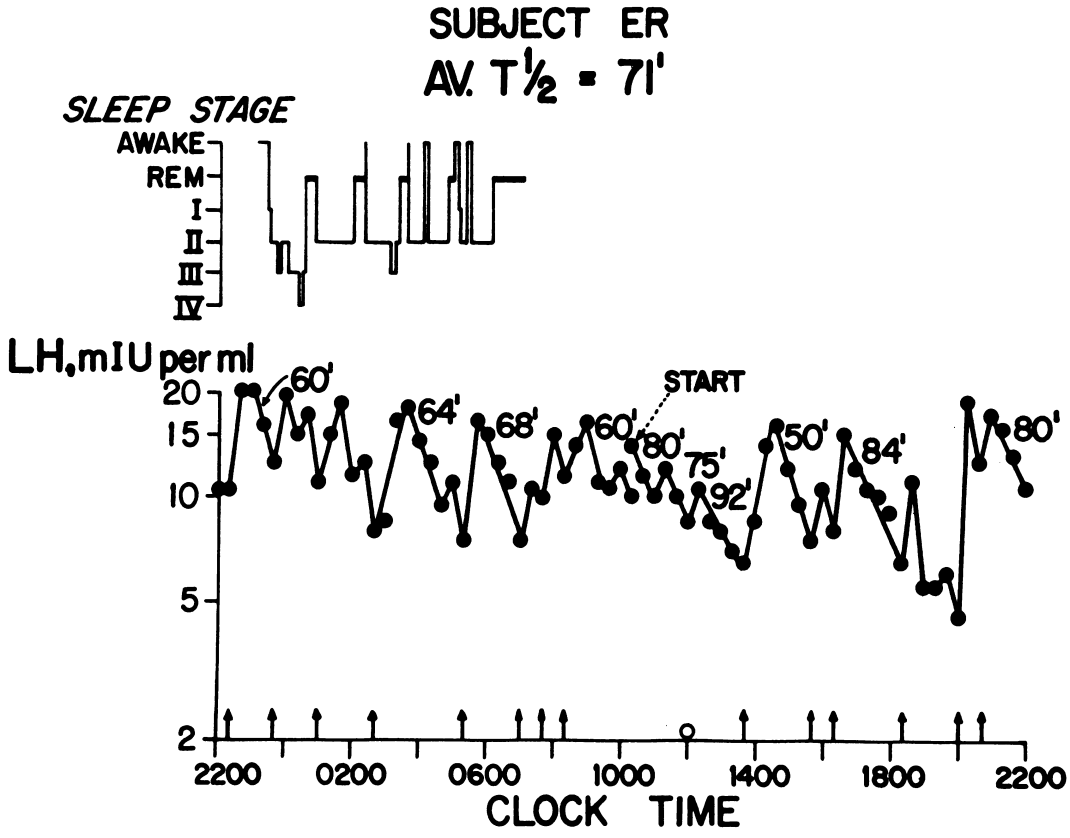

FIGURE 4. The 24-hour LH pattern in a normal adult man plotted on semi-log coordinates. The estimated half-lives are shown on the descending limb of LH secretory episodes where 3 or more successive declining points were available. The average of the individual half-lives in this subject was 71 minutes.

quent plasma sampling studies are completed and urinary LH measurements are made.

Summary

The ontogeny of LH secretion has been described. LH production probably begins in the fetus as early as the 2nd month of gestation, reaching its highest level during the second trimester. Increased levels have been demonstrated between the 1st–12th months of life, although their biologic significance is unknown. The prepubertal period is characterized by low levels until the onset of augmented secretory activity during sleep which occurs sometime in "late" prepuberty and persists until the completion of sexual maturation. Adult men show episodic LH secretory activity of equal magnitude during sleep and waking periods. After age 50, there is a gradual increase in LH levels, although these data are based on single plasma determinations. Since there are no published LH data in patients with BPH, a statement about changes in this hormone associated with this disorder must await future investigations. These investigations should be designed to take into account the following: a) the episodic nature of LH secretion; b) possible changes in the metabolic clearance rate of LH with aging, and c) an appropriate control population matched for age, sex and "non-specific effects of illness".

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DISCUSSION

R. HORTON: I was delighted to hear your comments about Bardin's work indicating that a reasonable approach to estimating mean LH secretion despite episodic secretion can be accomplished by sampling through 3 hours. Of course, another approach would be constant withdrawal and integration of the data; however, there is also a third way, and that is to sample with as few as 6 or 8 values throughout the 24 hour period. That is a reasonable way to estimate the area under the curve. There may be an inherent fallacy evaluating the relationship between LH and testosterone in the elderly male in which one would infer that because the testerone levels are low LH levels should be high. This is because LH feedback is derived both from testosterone and its conversion product, estradiol, which is formed both in peripheral sites and in the hypothalamus. Until we understand the peripheral conversions, particularly in the central nervous system, I am not sure we can make vadid comparisons based upon the younger individual.

S. G. KORENMAN: In regards to using urine for gonadotropin secretion, Hanson and Ross have observed a good correlation between the first morning urine and 24 hour integrated plasma gondaotropin using an acetone precipitate radioimmunoassay method. In their hands it is an excellent analysis with a 98% correlation.

G. WILLIAMS-ASHMAN: Are the changes in LH levels in pubertal boys related to light in any way? Have you ever looked at congenitally blind boys?

R. M. BOYAR: No. Not in congenitally blind boys, but we have looked at normal boys who have had sleep reversed or delayed and showed that augmented LH secretion is related to sleep and not the time sleep occurs. We have blindfolded pubertal boys during waking so that they had no light perception and were unable to initiate augmented LH secretion. So, it is clearly a sleep related event and not due to changes in the light-dark cycle.

J. GELLER: I think the studies showing a relatively low testosterone level with LH levels that were not elevated is in keeping with other data in the literature concerning LH responses to clomiphene in elderly men. It was reported a number of years ago that some men in the BPH age group have a rather poor response to clomiphene in terms of LH rises. We also did studies of testicular reserve in these patients that indicated a diminished but unequivocal response of the testes to HCG. I think that LH may be impaired in this age group.

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EFFECT OF AGE ON THE SERUM LH AND FSH RESPONSES TO GONADOTROPIN-RELEASING HORMONE

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Introduction

Interest in the serum LH and FSH responses to gonadotropin-releasing hormone (GnRH; also called LH-releasing hormone, LH-RH) derives largely from more general interest in whether there is any change in gonadal function with age in men. In studies initiated prior to the availability of synthetic GnRH, Vermeulen et al. (1) and Stearns et al. (2) demonstrated that serum testosterone concentrations decreased gradually and variably with increasing age, beginning after the age of 50. Serum LH concentrations in the men in the latter study increased gradually with increasing age, suggesting that the hypogonadism was due to a primary testicular abnormality. The availability of synthetic GnRH has permitted the study of stimulated LH and FSH secretion, in addition to basal secretion. At least four studies of the LH and FSH responses to synthetic GnRH administration in the male senescence have now been completed.

Results of Four Studies

Rubens et al. (3) determined the serum LH and FSH responses to an acute 200μg i.v. dose of GnRH in 60 Belgian men, 30 normal men under 50 years old and 30 normal men over 65 years old. Their results are summarized in Table I. The older group had significantly ($P < 0.001$) lower serum testosterone and dialyzable testosterone concentrations than the younger group. The basal serum LH and FSH concentrations were elevated in the older group compared to the younger group, demonstrating a moderate degree of primary hypogonadism in the men over 65. The serum LH and FSH responses to synthetic GnRH were also greater in the older group in regard to their absolute magnitude. Relative to the basal LH and FSH levels, however, the serum LH and FSH responses were less in the men over 65.

Hashimoto et al. (4) determined the LH and FSH responses to an acute 100μg i.v. dose of GnRH in 33 normal Japanese men in three age groups, 18–29, 30–49, and 50–70 years old. Their data is summarized in Table II. Serum testosterone levels were not determined, so the degree of hypogonadism that occurred with increasing age is not known. The basal LH and FSH concentrations in the oldest group, however, were approximately double those in the two younger groups, suggesting that the oldest group had a moderate degree of primary hypogonadism. Even though the basal levels were greater in the oldest group, the serum LH and FSH responses to GnRH were approximately the same in the oldest group as in the two younger groups, so that the LH and FSH responses to GnRH relative to the basal concentrations were lower in the oldest group.

Snyder, et al. (Submitted for publication) determined the LH and FSH responses to an acute 250 μg i.v. dose of GnRH in 42 normal men in the United States. There were 14 men in each of three age groups, 20–39, 40–59, and 60–79 years old.

Supported in part by USPHS grants RR 40 and TO 1 AM 95649

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TABLE I—Results of Rubens et al. (3) on the effect of age on the serum LH and FSH responses to a 200μg i.v. dose of GnRH

	Age Group yrs <50	>56
	30 [b]	30 [b]
Testosterone, ng/dl	492	281
Dialyzable Testsoterone, ng/dl	2.3	1.3
Basal LH, mIU/ml	13.0	24.1
Increase in LH at 60 min, mIU/ml	69.1	78.3
Increase in LH at 60 min/Basal LH [a]	5.3	3.2
Basal FSH, mIU/ml	5.6	15.8
Increase in FSH at 60 min, mIU/ml	11.1	19.8
Increase in FSH at 60 min/Basal FSN [a]	2.0	1.3

[a] ratios calculated from the authors' data
[b] Number

TABLE II—Results of Hashimoto et al. (4) on the effect of age on the serum LH and FSH responses to a 100 μg i.v. dose of GnRH

	Age Group yrs 18–29	30–49	50–70
	8 [b]	14 [b]	11 [b]
Testosterone, ng/dl	--	--	--
Dialyzable Testosterone, ng/dl	--	--	--
Basal LH, mIU/ml	7.1	7.6	18.0
Maximum increase in LH, mIU/ml	85.8	83.6	83.0
Max increase in LH/Basal LH [a]	11.3	11.0	4.6
Basal FSH, mIU/ml	7.3	7.5	13.6
Maximum increase in FSH, mIU/ml	8.3	9.5	9.8
Max increase in FSH/Basal FSH [a]	1.14	1.27	0.72

[a] ratios calculated from the authors' data
[b] Number

These data are summarized in Table III. Serum testosterone concentrations did not change significantly with increasing age in these subjects, but the dialyzable testosterone concentrations were significantly lower with increasing age (20–39, group vs. 60–79, $P < 0.01$). The basal LH concentration was only slightly higher ($P < 0.05$) in the 60–79 year-old men than in 20–39 year-old men, and the serum FSH concentration was not significantly higher in the older men. The serum LH response to a GnRH was slightly lower, in absolute magnitude, in the oldest group compared to the youngest, and was significantly ($P < 0.05$) less relative to the basal concentration. The serum FSH response to GnRH

TABLE III—Results of Snyder et al.[a] on the effect of age on the serum LH and FSH responses to a 250 μg i.v. dose of GnRH

	Age Groups yrs 20–39	40–59	60–79
	14 [c]	14 [c]	14 [c]
Testosterone	703	605	608
Dialyzable Testosterone	21.7	16.0	14.3
Basal LH	7.4	9.6	10.8
Maximum increase in LH	53.7	54.3	37.9
Max increase in LH/Basal LH	7.3	5.7	3.5
Basal FSH	9.2	9.6	10.1
Mean [b] increase in FSH	3.0	2.9	1.1
Mean increase in FSH/Basal FSH	0.33	0.30	0.11

[a] Snyder, P. J., Reitano, J. F. and Utiger, R. D., Serum L. H. and FSH responses to synthetic gonadotropin-releasing hormone in normal men, submitted for publication.
[b] The "mean Δ FSH" is defined as the mean of the 30, 60, 90, 120, 150 and 180 min increments above the basal concentration for each subject.
[c] Number

was significantly less in the oldest group compared to the youngest, both in terms of absolute magnitude of response and relative to the basal level.

Haug et al. (5) determined the serum LH and FSH responses to an i.v. bolus dose of 100μg of GnRH of 50 normal Norwegian men, 20 to 89 years old. The total serum testosterone concentration was less in the nine 80–89 year old men (mean ± SEM, 390 ± 44 ng/dl) than in younger men, but the levels in 60–69 (740 ± 90 ng/dl) and 70–79 year old men (610 ± 60 ng/dl) were not different from those in 20–39 year old men (660 ± 60 ng/dl). Serum dialyzable testosterone concentrations were not determined. Neither basal serum LH nor FSH concentrations changed with age. The serum LH and FSH responses to GnRH, however, decreased with increasing age, both in absolute magnitude and relative to the basal level.

Discussion

When LH and FSH responses to GnRH stimulation are considered relative to their respective basal concentrations, elderly men have smaller LH and FSH responses than do young men in all four studies. The advantage of considering the LH and FSH responses relative to their basal concentrations is that it permits the detection of impaired LH and FSH secretion in the presence of primary hypogonadism. Primary hypogonadism alone results in elevated basal LH and FSH concentrations and supranormal LH and FSH responses to the

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administration of exogenous GnRH (6,7). The ratio of the maximum LH/basal LH concentrations, however, is normal or elevated in mild hypogonadism, as shown by Mecklenburg and Sherins (8) and confirmed by us (unpublished observations). A variable degree of primary hypogonadism in elderly men was found in the four studies reported here, ranging from none in the subjects of Haug et al. (5), to a slight degree in the subjects of Snyder et al., and to a moderate degree in the subjects of Hashimoto et al. (4) and of Rubens et al. (3). The absolute magnitude of the LH and FSH responses to GnRH in these studies varied directly with the degree of primary hypogonadism. The ratio of the maximum/basal concentrations of LH and FSH, on the other hand, was uniformly lower in older men than in younger men in all four studies. This analysis suggests what whatever degree of primary hypogonadism is present in the male senescence, LH and FSH responses to GnRH are generally impaired as well.

The reason for the impaired LH and FSH responsiveness to GnRH in the male senescence is not known. Possible explanations include inhibition of LH and FSH secretion by the elevated serum estradiol concentrations that have been shown to occur with increasing age (3) and a decrease in the LH and FSH secretory capacity of the gonadotrophs with increasing age. It may be relevent that the thyrotropin response to synthetic thyrotropin-releasing hormone is also diminished in the male senescence (9).

Summary

Four studies of the effect of age on the LH and FSH responses to the administration of synthetic gonadotropin-releasing hormone are reviewed. These studies show different degrees of primary hypogonadism in the male senescence, from none to moderate. All of the studies, however, show a decrease in the LH and FSH responsiveness to GnRH relative to basal LH and FSH concentrations in the male senescence.

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DISCUSSION

A. VERMEULEN: I don't think there's much difference between the results we reported and those reported in the slide. We studied a younger population. The purpose of the experiment was just to investigate whether GNRH was able to increase LH secretion. I think it is evident that GNRH stimulation results in an increased LH secretion.

P. J. SNYDER: Yes, GnRH is able to increase LH secretion, but the percentage increase seems to be somewhat inappropriate for the basal LH level.

O. HECHTER: How does one define hypogonadism? I ask this question because there is such a thing as physiological reserve. We have the ability to produce more hormone than we may need to maintain a particular function. Now are you relating the differences observed to a statistical deviation from a mean or to a clear functional deficiency?

SNYDER: I defined it very narrowly as a decrease in either the total testosterone concentration or the dialyzable fraction. The dialyzable fraction was measured in only two of these four studies.

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S. G. KORENMAN: The data do not convince me that this is true hypogonadism. Second, the criteria for patient selection, while physiologically apt, does not take into account all the possible factors involved. There is at least one study I recall in which elderly males were found to fall into a bivariant population with regard to serum total testosterone. There was a group with essentially normal serum testosterone and a group with reduced serum testosterone. A selection process might take place prior to study in which one would assume that those with low testosterone were hypogonadal. What was *the splay* in your own techniques, and what was the splay in other people's studies?

SNYDER: Only mean levels were reported in the three other studies. In our selection process we tried to be careful to eliminate people with specific diseases and specific medication that might possibly have altered the hypothalamic-pituitary-testicular axis. In other words, we tried to obtain people who were healthy. On the other hand, sexual performance was not a criterion for selection. And we did not exclude people on the basis of prior knowledge of their serum testosterone levels.

M. B. LIPSETT: The values for free testosterone are not "hypogonadal", and many normal young men have similar free testosterone values; thus by definition I don't think this can define a hypogonadal state. In other words, full virilization can occur with a plasma testosterone of 300 ng/dl and with similarly low free testosterones. Among the important variables that we don't study too often are those that can effect the central nervous system. Although there are many conflicting data about CNS effect on the Leydig cell, it is clear that it does something in the primate, in the rat and in man. To compare old men without saying whether they came from an old soldiers home on the one hand or upper middle class on the other would be, I think, very dangerous in looking at the hypothalamic-pituitary axis.

SNYDER: Our subjects all appeared to lead active lives. None was a resident of a soldiers home or nursing home. As to whether these subjects are actually hypogonadal, I wouldn't claim that any of them are severely hypogonadal, because their testosterone levels just aren't that low. On the other hand, as a group the oldest group definitely has a lower dialyzable testosterone concentration than the youngest group. That's not severe hypogonadism, but it is hypogonadism relative to the youngest group.

H. BRENDLER: I would like to return to the response to GnRH, which you characterize as inappropriate in the older male. Could this not be explained on the basis either of the fact that the older male is already producing LH or possibly due to the decreased sensitivity in target tissues in the older male.

SNYDER: I think that the LH response to GnRH is inappropriate in relation to the basal level of LH. In other words, in any young man who has exclusively primary hypogonadism, i.e., testicular disease with an elevated basal LH level, the absolute magnitude of the increase in LH in response to GnRH is much greater than the increase seen in normal subjects. An increased LH response to GnRH does not occur, however, in the older man, even though they have mildly elevated basal LH levels.

M. HARMAN: I would like to speak on the question of what is hypogonadism. Someone mentioned the fact that there is very wide distribution of values. In the studies I have reviewed, there is a very wide range. Some elderly men have testosterones well over 500 nanograms per deciliter, and some of them have levels in the female range, around 30–40. The real question it seems to me is that if you characterize these two groups of men, can you say anything about functional correlates—clinical, psychosexual and so on? Looking at mean testosterone is not too helpful.

SNYDER: Certainly in large populations that is quite appropriate. A more sophisticated analysis would be inappropriate for the number of subjects we studued. In our study there were 42 subjects, 14 in each of three age groups, and there was not so wide a distribution as you mentioned. None of the subjects had levels in the so-called female range.

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PROLACTIN AND AGING

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Introduction

Prolactin has only recently been identified as a separate human pituitary hormone. The sequel to this achievement was the development of a homologous radioimmunoassay for human prolactin (hPRL). The sensitivity and specificity of the prolactin assay have been useful in producing data that alter and extend our limited understanding of the physiologic factors that influence prolactin secretion in man. In this presentation, we propose to consider several variables that are of importance in assessing serum prolactin concentrations. Among these factors are 1.) the heterogeneity of prolactin in plasma; 2.) two types of fluctuations in serum prolactin, namely, diurnal or circadian and episodic; 3.) the effects of age, sex and stress on serum prolactin. Pharmacologic agents that increase or decrease prolactin secretion have been identified, and these have proved helpful in evaluating the hypothalamic pituitary prolactin axis in various conditions including old age. Finally, we shall briefly present evidence that, in rats, there are specific prolactin binding sites in particulate fractions from several tissues including the prostate and that the number of binding sites are modulated by hormonal changes and age. On the basis of these observations, we suggest that both the concentration of serum prolactin and the tissue receptors for prolactin may be important in determining the biological effect of this hormone.

Prolactin secretion is controlled by the tonic release from hypothalamic centers of prolactin inhibiting factor (PIF) which is transported via the portal capillary system down the pituitary stalk to act on the prolactin cell to inhibit its secretion. The nature of PIF and its mechanism of action are still obscure. Currently, there is some debate whether PIF might be dopamine rather than a novel peptide (1).

Before reviewing the factors that affect prolactin secretion (listed in Table I), it is worth noting that several investigators have reported on the heterogeneity of prolactin in serum (2,3). In these studies, serum samples from both normal subjects and samples from patients with prolactin-secreting pituitary tumors were fractionated on Sephadex, and the immunoreactive components were identified by radioimmunoassay. In normal subjects, between 10 and 30% of the immunoreactive prolactin was of larger molecular weight, having an elution volume that indicated a component 2 to 3 times

TABLE I—Physiological Variables Influencing Serum Prolactin

1. HETEROGENEITY	"Big Big" "Big" "Little"
2. FLUCTUATIONS	(a) Circadian (Sleep Related) (b) Episodic
3. AGE	
4. SEX DIFFERENCES	Male Female —Menstrual cycle (?) —Pregnancy —Lactation
5. STRESS	

This work was supported by the Medical Research Council of Canada, the U.S. Public Health Service Child Health and Human Development (Grant 5 RD1-HDO7843-02) and the National Cancer Institute (Contract NO1-CA-43250).

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the size of the principal monomeric form. In another study, it was found in serum from a patient with a prolactin-secreting tumor that most of the immunoreactive component appeared in the void volume indicating a MW 100,000. In both studies, radioimmunoassay of serial dilutions of this "big" prolactin indicated parallelism with the monomeric form. However, by radioreceptor assay in one study (4), the concentration of "big" prolactin estimated by radioreceptor assay was considerably less than that determined by radioimmunoassay, indicating that perhaps not all the immunoreactive components are biologically active.

A circadian periodicity in the concentration of pituitary and serum prolactin has been established in rats. Diurnal variations and episodic oscillations in serum PRL concentrations also exist in humans (5,6). Serial measurements reveal marked elevations 4–5 hours after the onset of sleep. The increase in prolactin is sleep-related and is not the result of an endogenous circadian rhythm. The circadian periodicity in prolactin secretion is maintained in women on oral contraceptives, after the menopause and in lactating women, but is not evident in women in the last trimester of pregnancy (5). In addition to the circadian variation, episodic fluctuations in prolactin levels in serum have also been reported, but the periodicity of these oscillations is not as well defined as it is for LH (6).

Prolactin secretion is stimulated by a number of different kinds of stress both in animals and man (7). The mechanism and the physiological importance of stress-induced prolactin secretion is unknown at present. However, it is important to bear this factor in mind in designing any large-scale study in order to minimize the effects of stress.

Fig. 1 depicts mean serum hPRL concentrations in humans at various stages of life as reported by different investigators. Aubert et al (8) in a recent study on the ontogenesis of human prolactin investigated serum hPRL in fetuses ranging in age from 2 months until term. Serum hPRL concentrations were 2.9 to 67 ng/ml with a mean of 19.5 ± 2.5 ng/ml from 90 to 160 days of fetal development and 200 to 500 ng/ml in late gestation. Serum levels in the full-term newborn are at about the same level

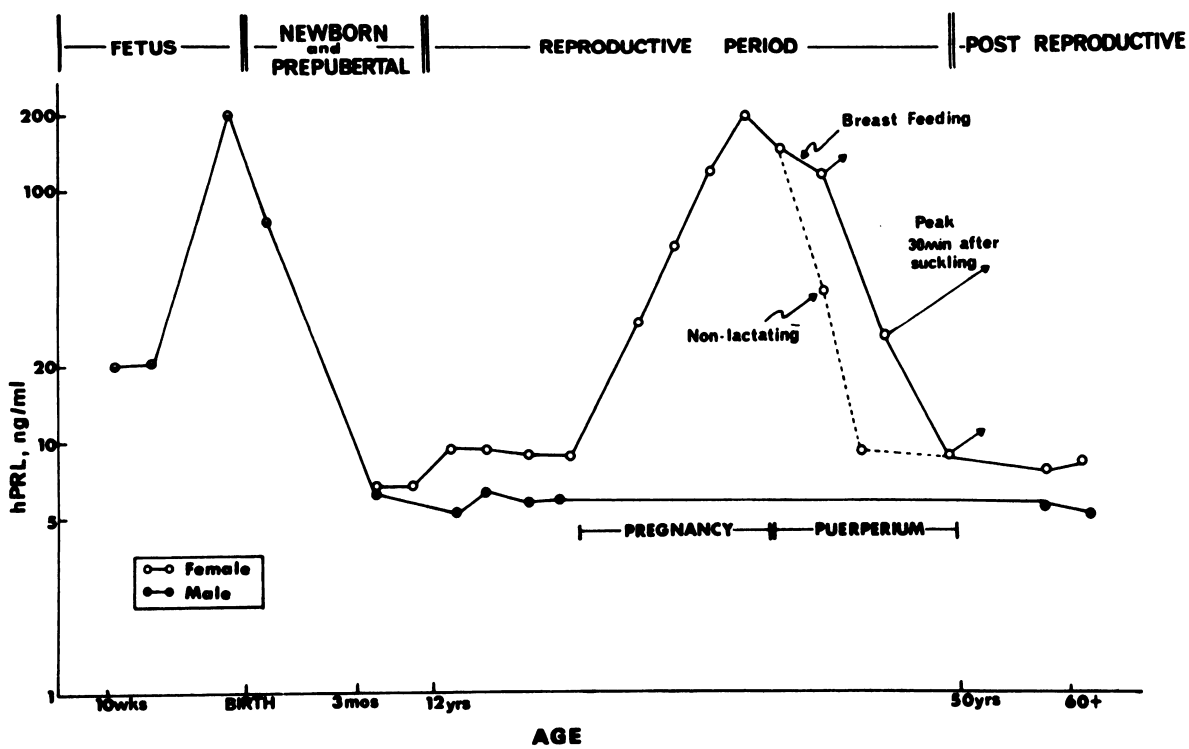

FIGURE 1. Serum prolactin levels in human. Fetal values are those reported by Aubert et al (8). See text for further details regarding the changes observed during pregnancy and the postpartum period.

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(200 ng/ml) being slightly lower than values in maternal serum at term. This elevation persists longer in premature infants than in full-term newborns, and it may be 3–6 months before normal adult levels are reached. Subsequently, in childhood the levels of prolactin in males and females are similar until puberty when, as a result of increasing estrogen secretion in females, a slight increase in prolactin levels are observed. When the range of values for adult men and women are compared, there is a great area of overlap, but, in most studies, a significantly higher mean prolactin concentration is found in women (8 ± 2ng/ml) than in men (6 ± 2ng/ml). In women Robyn et al (5) have reported elevated plasma prolactin levels at midcycle in a group of 28 normally menstruating women, but in other studies this has not been confirmed (6). There appears to be better agreement on the fact that in the luteal phase there is considerably more variation in prolactin values than in the follicular phase.

During pregnancy in humans elevated levels of prolactin first appear in maternal serum at 8 weeks gestation and continue to rise until term when a mean value of 200 ng/ml is reached. In the first trimester, amniotic fluid concentrations of prolactin as high as 10,000 ng/ml have been found at a time when maternal and fetal serum prolactin values range between 20 and 50 ng/ml. The amniotic fluid prolactin levels decrease gradually and at term reach a concentration of 1000 ng/ml which is still five to eight-fold greater than the mean maternal serum value. Amniotic fluid prolactin appears to be immunologically, electrophoretically and chromatographically identical with pituitary prolactin (5). The exact source of amniotic fluid prolactin remains unknown, but preliminary evidence suggests that it may be produced by the chorion. In the postpartum period in women who do not breast-feed, serum prolactin levels return to values found in nonpregnant subjects in 2–3 weeks. In women who breast-feed, Tyson et al (10) have described three types of prolactin response to suckling at different times in the postpartum period.

Few studies have been reported on prolactin secretion in animals and humans with advancing age. Clemens and Meites (11) compared the hypothalamic and pituitary functions in 21-month old rats in constant estrus with those of 3 month old cycling rats on the day of estrus. They found that hypothalamic prolactin inhibitory factor levels were similar in both groups, but pituitary prolactin concentration was significantly greater in the old rats. Furthermore, as cited by Clemens and Meites (11), unpublished observations by Voight and Wuttke indicated that serum prolactin values in old constant estrus rats as measured by radio-immunoassay are about as high as in young adult cycling rats on the day of estrus. It was suggested that the constant estrus observed in many old female rats is the result of a fundamental change in hypothalamic control of pituitary FSH, LH and prolactin secretion.

In humans, few studies have been reported concerning serum prolactin levels in aging subjects. In women prolactin levels remain largely unchanged with the advent of the menopause.

Perhaps the most useful information regarding the effect of sex and aging on prolactin release is provided by the interesting study of Jacobs et al (12) who examined the prolactin response to thyrotropin releasing hormone in normal subjects of both sexes and of different ages. In their study, the response to TRH of men and women was compared in 3 age groups. The increase in prolactin was greater in women than in men in all three age groups (Fig. 2). This difference was roughly twofold and was highly significant in the young (56.8 ± 4.8 (SEM) vs. 27.9 ± 1.9 ng/ml $p<0.001$) and middle-aged (74.2 ± 9.9 vs. 30.0 ± 3.3 ng/ml $p<0.001$) subjects. In the elderly subjects the differences between the sexes was smaller and was not statistically significant (45.7 ± 7.0 vs. 28.5 ± 6.5 ng/ml $p>0.05$). Since the PRL response to TRH in prepubertal children is similar in males and females and comparable to the responses in adult men, the increased PRL response of adult females appears to be established during or after puberty and persists thereafter. Among subjects of the same sex PRL responses were comparable in the three age groups, except that the response of the elderly females was somewhat lower than that of the younger groups, being significantly lower only in comparison to the response of the middle-aged females and not significantly lower than that of the young females so that the authors considered it difficult to attribute great importance to this finding. In both sexes, the variation in PRL responses increased progressively with increasing age. The coefficient of variation of the mean maximum increase in PRL in the elderly men and women was respectively three-fold and two-fold greater than in the young subjects.

In preliminary studies of the effect of chlorpromazine in aged female schizophrenics who were on maintenance doses of phenothiazines, we noted no increase in basal prolactin from control subjects not on drugs. Most patients on phenothiazines of course have elevated basal serum prolactin concentrations (13). In addition, after chlorpromazine was administered serum hPRL actually fell instead

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of increasing (Fig. 3). These results are preliminary and need to be extended to determine whether this paradoxical response is peculiar to schizophrenics, old age, chronic long term therapy with phenothiazines or a combination of all three.

There are a number of areas which require further investigation. We do know whether the heterogeneity of prolactin in serum varies with age. We do not know whether circadian and episodic secretions persist in old age. There is only very limited information on the effect of stress and pharmacologic agents on serum prolactin in aging males. It is obvious that large gaps in our knowledge of prolactin secretion remain.

To assess the overall biological effect of a hormone, at least two factors must be known: hormone concentration and target tissue responsiveness. To date, much of our effort has been concentrated on measuring hormone levels, but within the past several years assessment of hormone receptors as an index of tissue responsiveness has received increasing attention. These experiments have demonstrated that specific hormone binding sites on plasma membranes are not static entities but are themselves under dynamic control regulated by a variety of factors including 1) age, 2) sex, 3) hormones, 4) dietary factors, etc.

In the case of prolactin, we have studied the ontogenesis (14), tissue distribution (15), and the effects of pregnancy (14), hormones (16,17,18) and inhibitors of protein synthesis (17) on prolactin binding sites in the liver. The results of some of these studies will be reviewed briefly. The prolactin receptor in female rat liver has been employed in some of these studies as a model system. The binding site has a high specificity and affinity for lactogenic hormones. As shown is Fig. 4, the receptor levels increase at the time of puberty in female rats and fluctuate with the various stages of the estrous cycle. Pregnancy causes a further increase in number of receptors. In male rats, treatment with estrogen leads to an induction of prolactin receptors

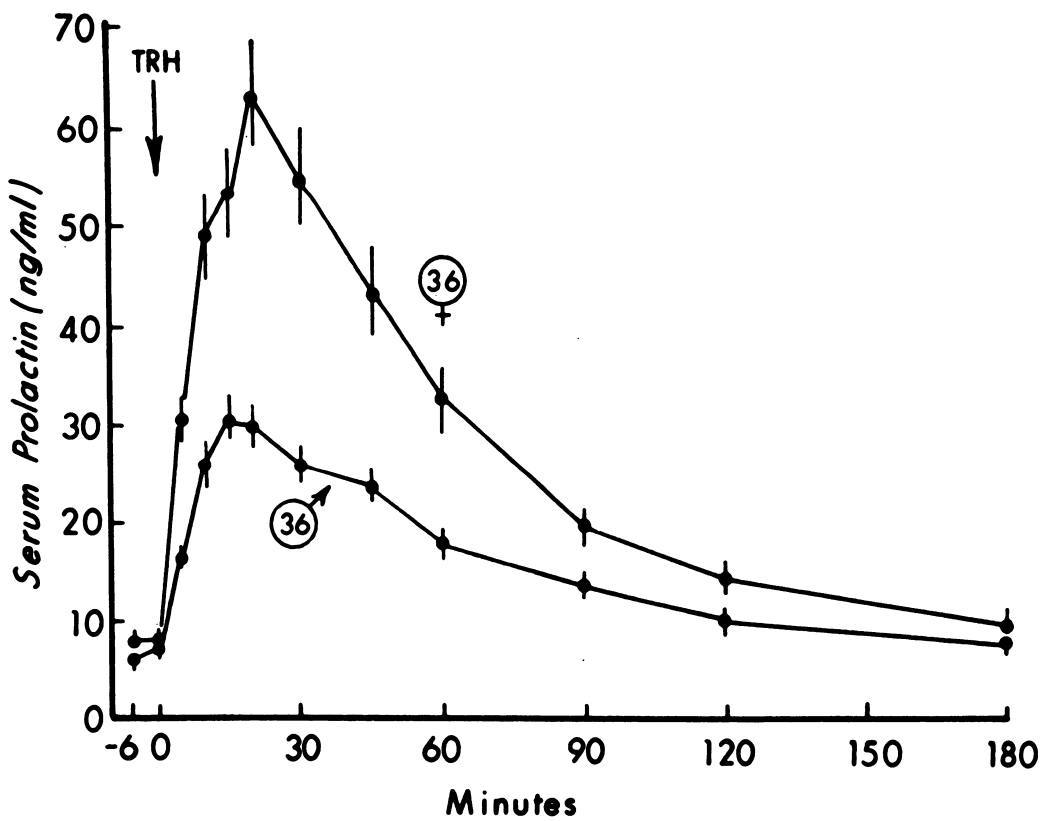

FIGURE 2a. *Upper Panel:*
Time course of the serum prolactin response to the intravenous injection of 400 μg of synthetic TRH in 36 normal men and 36 normal women. Each point represents the mean of the 36 values for each sex: vertical bars indicate one standard error of the mean. Taken from Jacobs et al (12) with permission.

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in the liver, so that the number of receptors exceeds that found in females. The pituitary gland appears to have a critical role in the induction of the receptor since hypophysectomized male rats are unresponsive to estrogen. Likewise, following hypophysectomy in female rats the prolactin receptors disappear rapidly as judged by the decrease in specific binding of ^{125}I-labelled prolactin to membranes. On the other hand, implantation of pituitaries into the kidney capsule of hypophysectomized female rats results in the secretion of prolactin and maintenance of prolactin binding sites and in hypophysectomized male rats induced high levels of prolactin receptors in the liver. The secretion of rat PRL by the ectopic pituitary presumably is the factor responsible for the induction and maintenance of the prolactin receptor (18). Finally, after a single injection of 200 μg cycloheximide, the lactogenic receptors in male rat liver is reduced by more than 90% within 3 hours and by 48 hours has returned to pretreatment levels. From these studies, several conclusions may be drawn: 1) that a certain hormonal environment is required for the maintenance or the induction of prolactin receptors; 2) that continuous active protein synthesis is essential for the maintenance of membrane receptors whose rapid turnover may have an important role in modulating hormone action.

We have discussed the regulation of the specific prolactin binding sites in liver. We wish to conclude by reporting that in male rat accessory sex glands, particularly in the prostate, similar mechanisms control specific prolactin binding sites. Not much is known about the role of prolactin in male animals. A number of effects of prolactin on testicular function and prostatic growth have been reported (19,20). In the light of this evidence, we examined whether specific PRL receptors could be identified in tissue homogenates and membrane preparations of male rat gonads and accessory tissues. In membrane

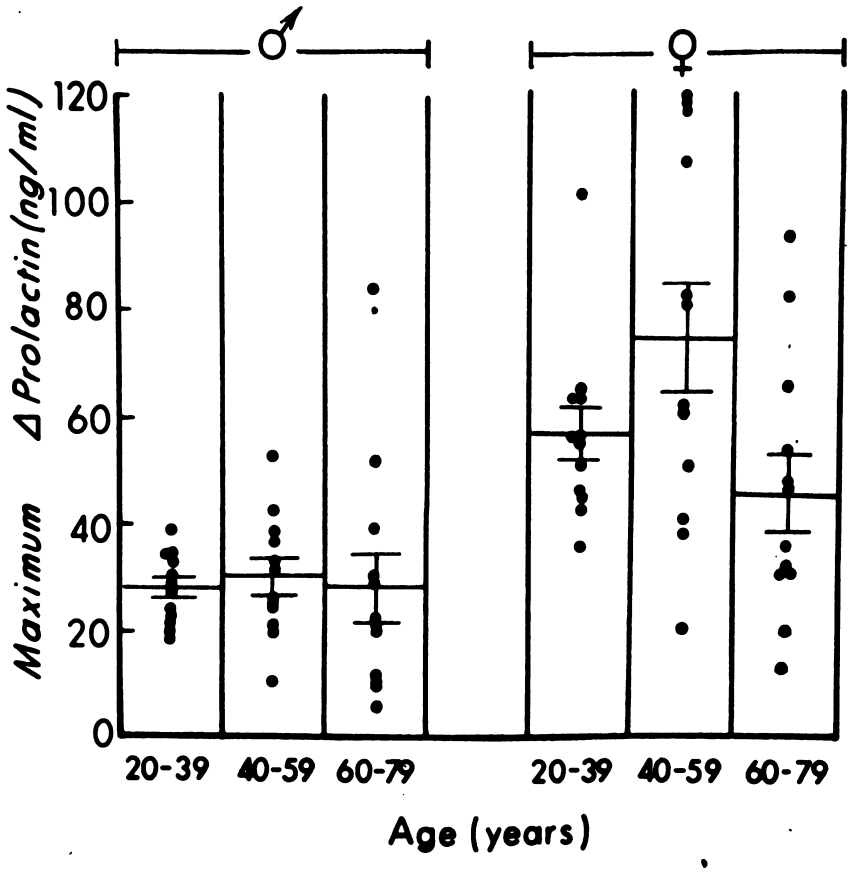

FIGURE 2b. *Lower Panel:*
The effect of age and sex of prolactin response to TRH. Each point represents the maximum prolactin in a single subject. The heavy horizontal lines indicate means, and the lighter solid lines indicate one standard error on either side of the mean. Taken from Jacobs et al (12) with permission.

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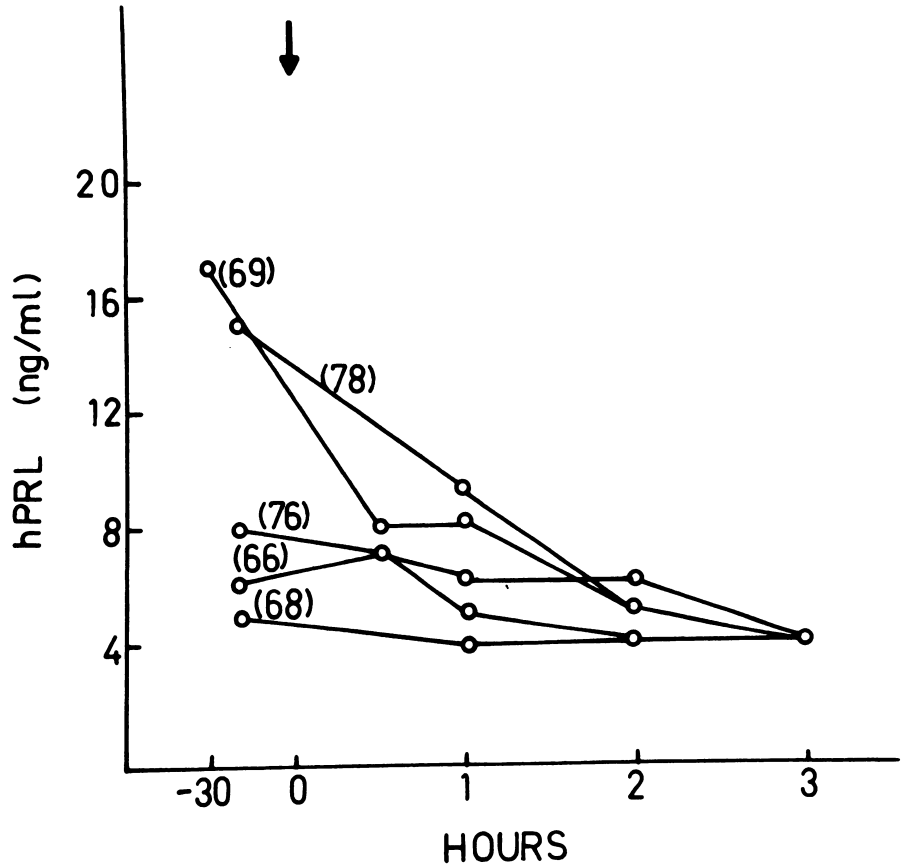

FIGURE 3. The response of 5 female schizophrenic subjects to the intramuscular administration of chlorpromazine. The age of each individual is indicated in parenthesis. In healthy, normal individuals, an equivalent amount of phenothiazine would lead to an increase of 25 ng/ml above the basal value between 1 and 2 hours.

preparations from adult rats, specific binding of ^{125}I-ovine PRL (oPRL) was found in the following tissues (expressed as % of ^{125}I-oPRL bound per mg protein): testis, 3.3; epididymis, 2.6; seminal vesicles, 11; and prostate, 57 (Fig. 5). The binding of ^{125}I-oPRL was inhibited only by excess unlabelled PRL and other hormones with lactogenic activity but not by hormones such as bovine growth hormone. Scatchard analysis of the binding of PRL to prostatic membranes indicated that the affinity and binding capacity of the PRL receptor in the prostate is similar to that previously reported for the rabbit mammary gland (Ka $3 \times 10^9 M^{-1}$). The effect of age and hormonal changes on binding was also examined (Fig. 6). In the testes, the low specific binding of PRL increased with age from 0.9% at day 21 to 3.8% at day 70. By contrast, in prostatic membranes, the highest specific binding (83%) was found in 20 day-old rats, and in 270 day-old rats binding decreased to 10%. The administration of estrogen caused a 50% reduction in the binding of PRL to prostatic membranes within 11 days, and an even greater reduction of 90% occurred 14 days after castration (Fig. 7). On the contrary, in liver membranes estrogen treatment (as previously reported) or castration produced at least a 20-fold increase in the binding of PRL.

In summary, specific binding sites for PRL have been identified in the testes and the accessory tissue of reproduction in the male rat with the highest number in the prostate. These results lend further support for the concept of a functional role of PRL in the prostate of the rat. It is evident that some of the same mechanisms that influence the number of specific binding sites for prolactin in rat liver are also operative in prostate.

Conclusion

In the light of evidence presented we believe that

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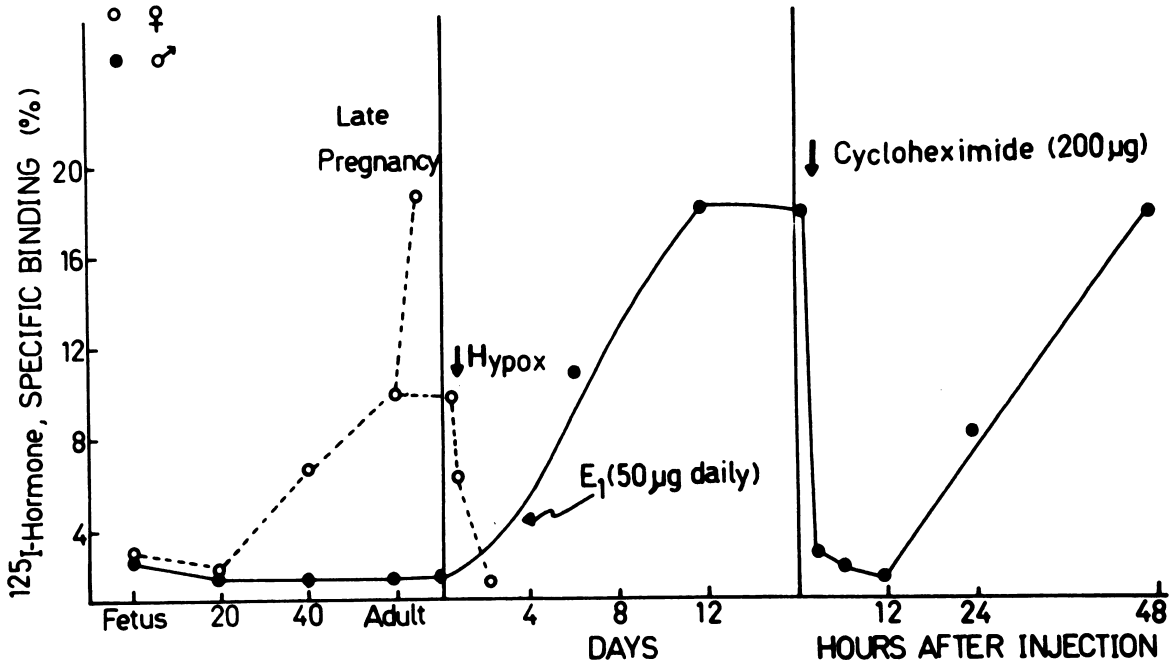

FIGURE 4. *Left Panel:*
Changes in specific binding of ^{125}I-PRL in liver membranes of male and female rats. For details of the study, see references 14–17. In prepubertal rats binding is low but similar in males and females. With puberty, the binding of prolactin increases in female liver, and there is a further increase in pregnancy.
Middle Panel:
Hypophysectomy leads to a rapid decline in specific prolactin binding sites in female liver, whereas estrogen treatment of the male rat leads to an induction of prolactin binding sites.
Right Panel:
Cycloheximide leads to a prompt and transient reduction in specific binding sites for prolactin (17).

when considering the role of prolactin in prostatic growth and function at different age periods, it will be necessary to consider not only serum prolactin concentrations but also prostatic tissue responsiveness under different conditions. It may well be that the latter is at least in part determined by the number of plasma membrane receptors for prolactin.

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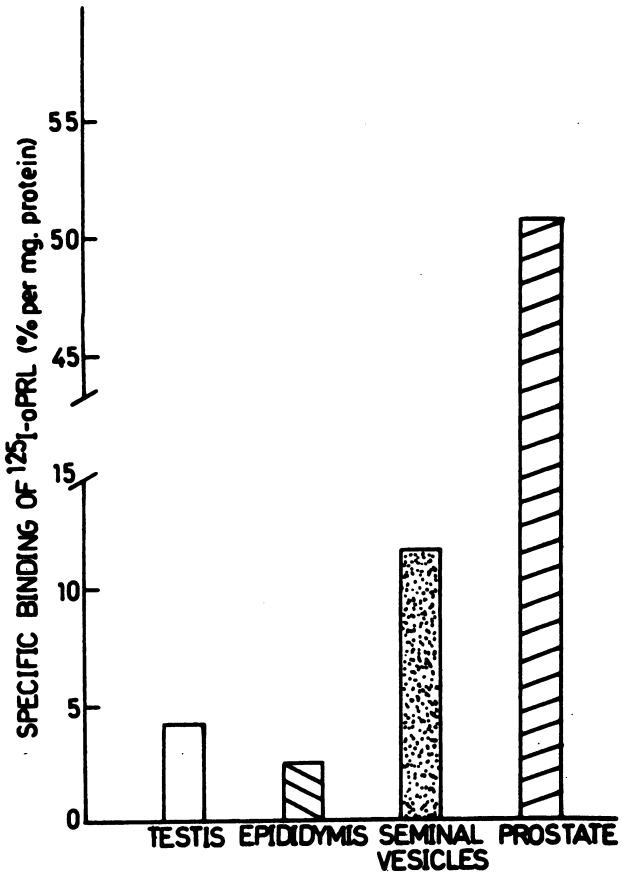

FIGURE 5: Specific binding of ^{125}I-oPRL to membrane preparations from the testes, epididymis, seminal vesicles and prostates of 100 day-old rats. Membrane preparations (450 μg) were incubated in duplicate with 100,000 cpm of ^{125}I-oPRL for 12 hours at 24°C with constant shaking. The buffer employed was 25mM Tris-HCl, pH 7.5, containing 10 mM $MgCl_2$ and 0.1% (w/v) bovine serum albumin. The final volume was 0.5 ml, and parallel incubations were performed in the presence of excess unlabelled oPRL (1 μg). The incubation was terminated by the addition of 3 ml of incubation buffer at 4°C, and bound and free ^{125}I-oPRL were separated by centrifugation at 3,000 rpm at 4°C for 20 minutes. The supernatant was discarded by decanting, and the pellet was counted. Specific binding was the difference between radioactivity (cpm) bound to the membranes in the absence of excess unlabelled hormone and that bound in its presence. The ordinate specific binding is expressed as a percent of the ^{125}I-oPRL bound per mg/protein.

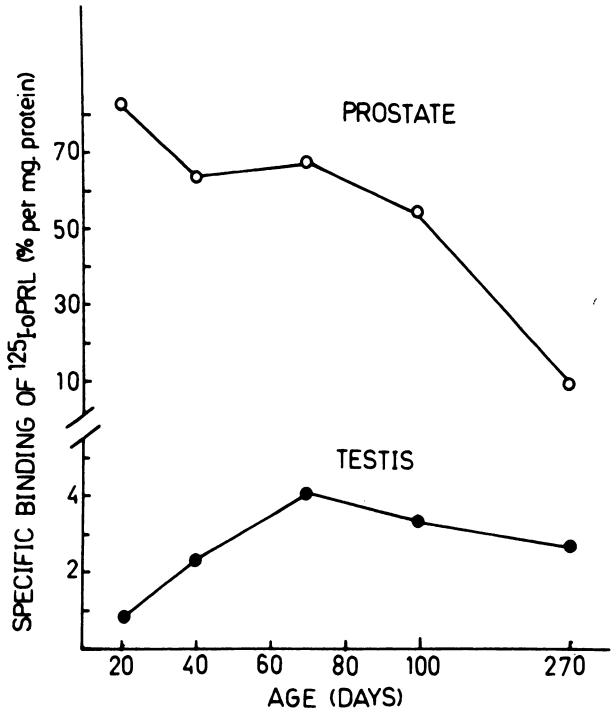

FIGURE 6. Effect of age on specific binding of ^{125}I-oPRL to prostatic and testicular membranes. Membrane fractions from prostates and testes of rats of different ages (10 rats in each age group) were used. The details of incubation conditions and calculation of specific binding are found in the legend to figure 5.

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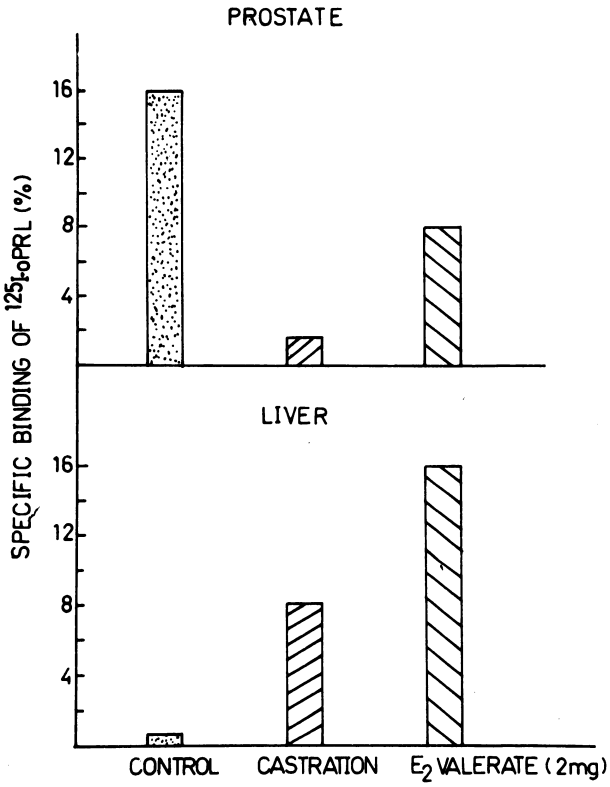

FIGURE 7. Effect of castration and estrogen administration of ^{125}I-oPRL specific binding to rat prostate (upper panel) and liver membranes (lower panel). Details of method are found in legend to figure 5. In the estrogen-treated group, 2 mg of estradiol valerate was administered as a single injection subcutaneously to 10 rats. In the control group, rats were injected daily with 0.5 ml of saline. Castration was performed surgically in 10 rats while the control group were sham operated. The studies were carried out approximately 10 days later.

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DISCUSSION

G. WILLIAMS-ASHMAN: When you are talking about rat prostate, to which lobe of the gland are you referring?

H. G. FRIESEN: The entire prostate.

WILLIAMS-ASHMAN: Then it is difficult to interpret your results because the response in some strains of rats to prolactin varies in different lobes. Perhaps Dr. Grayhack would like to elaborate on this.

J. T. GRAYHACK: The response of the dorsolateral lobe, particularly the lateral, is more definite and more easily reproduced and recognized as compared to the ventral lobe.

L. LIPSETT: I wanted to ask you if there are data in other species. I raise the question because we know that in the rodent prolactin is active on the testis and corpus luteum and also affects prostate growth. Do we know anything about any other species with respect to binding, for example?

FRIESEN: We have not looked at other species.

M. TANNENBAUM: We have recently obtained some antiserum to prolactin from Dr. A. Frantz and by using two immunohistochemical techniques (immunofluorescence and immunoperoxidase) have looked at prostatic tissue but were not able to get any localization of ovine prolactin. However, when we examined the pituitary of humans, there was localization by these two techniques. Thus, with the antiserum preparations that we have in our hands, the human prostate does not contain binding sites for prolactin.

FRIESEN: Could you tell us the minimal amount of hormone that can be detected by your technique?

TANNENBAUM: This is the work that Drs. E. Zimmerman, K. C. Hsu, A. Frantz and myself have reported. By means of these immunological techniques we can detect in one cell 60–80 molecules of neurophysin, which is the carrier protein for oxytocin. With these new immunological techniques, one can determine the site of cellular localization and detect minute quantities.

O. HECHTER: When you speak of specific binding of prolactin to tissue, there is an implication that this binding is functionally important with respect to hormone action. What do you really mean by specific binding, and if there is specific binding, does this necessarily mean that there is a functional role for this binding?

FRIESEN: I don't think that it necessarily follows, but for the moment that has been our assumption. It is a subject that could be debated extensively, but until proved otherwise we believe that this is a reasonable hypothesis.

HECHTER: The converse may be true. There is a substantial question in my mind as to the physiological role of prolactin in the male.

FRIESEN: There is reasonable evidence that pro-

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lactin in the male rat influences renal function and that prolactin acts in a synergistic manner with testosterone to promote prostate growth. There are also clinical data that prolactin may have a role in testicular function in man. In one male subject that we studied with Dr. Refetoff at the University of Chicago, the patient had elevated prolactin levels and as I recall testosterone levels around 100 nanograms per deciliter. When this man was treated with CV-154 and prolactin was lowered within several weeks, testosterone levels rose ten fold. Subsequently, when CB-154 was withdrawn and prolactin levels rose, within three weeks testosterone levels were again decreased. So, I think that there is reasonable evidence in the case of the human male that prolactin has a role to play.

L. M. FRANKS: Dr. Friesen, could you clarify one thing for me. Did you say in the liver binding studies that prolactin appeared to induce more binding sites?

FRIESEN: Yes.

FRANKS: In the castrated prostate you have less androgen binding, and I wonder if there is any relationship there.

FRIESEN: The binding in each tissue varies depending upon the hormonal milieu. In the case of prolactin and growth hormone certainly, it appears that hormonal changes influence binding differently in different tissues and differently in different species. Some of the data at this point are hard to interpret in terms of biological significance, but we must proceed on the notion that these changes are of some importance.

FRANKS: Could you speculate as to what function prolactin might be having in the liver?

FRIESEN: There is one study to suggest that prolactin may induce estrogen receptors in liver tissue. In addition, prolactin has been shown to increase ornithine decarboxylase 10 to 20 fold.

J. A. THOMAS: Your data indicated that the prostate had a higher binding than the seminal vesicle. This would be somewhat different than some of our preliminary studies in which higher amounts were found in the seminal vesicle. In our experiments the ventral lobe was used which has not been particularly responsive to the so-called synergistic effect of prolactin and testosterone. I think it would be important to do a survey of the lobes of the rodent prostate to determine whether or not the prolactin receptor is higher in those lobes that show a synergistic effect to testosterone, namely the dorsolateral lobes.

FRIESEN: I agree. (Since this conference, we have demonstrated that the dorsolateral lobe is the one that contains prolactin receptors.)

R. F. GITTES: I would just like to review briefly the quandary in dealing with prolactin. We have the observations of Dr. Grayhack, but the rats don't get BPH. We have assays in animals, and again the animals that we have assays for are also not BPH candidates. We tried to utilize the other aspect of prolactin which is available to us, and that is the fact that it is supposed to be elevated if you have a transplant from the pituitary. We examined the preparation of the normal animals with multiple pituitary ectopic transplants to see what that would do to the prostate. We have seen no effect on prostate growth in spite of presumably normal testosterone levels and no effect on the structure of the prostate. But again, the quandary; we've been working with rodents that don't get BPH, and the study will have to be performed with the proper combination of species and techniques to determine the role of prolactin in the pathogenesis of BPH.

GRAYHACK: Did you say that you got no effect with the transplanted pituitaries?

GITTES: With additional transplants in animals who already had intact pituitaries this was providing only more prolactin. We observed no histologic effect, and we did measure elevated prolactin in animals treated as long as 8 months.

Y. TOPPER: In extension of Dr. Hechter's comments, there is an example in which binding of a hormone to a cell does not lead to biological response. Mammary cells from virgin mice are insensitive to insulin, but mammary cells from pregnant mice are sensitive. Yet the two types of cells bind insulin equally well. The binding in the case of virgin cells does not lead to biological response, while the binding in the case of pregnant cells results in a variety of responses.

HECHTER: Oh, there are many examples of the dissociation between specific hormone binding and biological action.

FRIESEN: I share Dr. Hechter's concern about the interpretation of specific binding. The most direct demonstration that the binding is in fact to a receptor would be to block the receptors selectively and to demonstrate that there has been a block of the function of prolactin. The approach that we have adopted is to attempt to purify the prolactin binding protein in rabbit mammary glands, to generate antibodies to these, and in turn to use the antibodies to block the binding and hopefully the biologic effect of prolactin in the mammary gland. This is the kind of evidence that would be helpful in proving binding is related to function. This demonstration would lead one to conclude that one is dealing with the biologically important binding site. This sort of thing has been done with the acetyl-

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choline receptor. It is of some interest parenthetically that the antiserum generated against the rabbit mammary gland prolactin receptors purified about 2000 fold selectively blocks the binding of prolactin not only in the mammary gland but also in prostatic tissue and in rat liver without affecting the binding of growth hormone. So, in that sense there is some additional evidence for specificity.

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TESTICULAR HORMONAL SECRETION AND AGING IN MALES

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A decrease in sexual activity with increasing age has been generally accepted as an established fact by the lay public, and ever since Brown Sequard's and Voronoff's experiments the role of a decreased endocrine function of the testes has been suspected.

However it is now evident that the increase in sexual vigor experienced by Brown Sequard after injection of aqueous or glycerol extracts of dog testes was essentially due to the psychological impact of this procedure, and until recently the reality of a decreased Leydig cell function in old age has been disputed. This is rather surprising since an important decrease in the urinary excretion of 17 ketosteroids with increasing age has been known for almost 20 years (1); an important decrease in adrenocortical secretion in old age has never been documented, and the decreased 17 ketosteroid excretion should logically implicate a decreased Leydig cell function.

In 1957 (2) Tillinger observed a significant decrease in Leydig cell mass in old age, and Hollander & Hollander (3) first reported a decrease with age in the testosterone level in human spermatic vein blood.

Subsequently however several authors (4–7) reported that plasma testosterone levels remain within the same range from adolescence until old age.

In 1966 (8) we reported a significant decline in urinary *testosterone-glucuronide* excretion with age from the 5th decade of life on, suggesting either a decrease in testosterone secretion or a modification in testosterone (T) metabolism. We observed also, albeit in a relatively small group of patients, a decrease in urinary testosterone production rate (9). Kent & Acone (5) reported a decreased metabolic clearance rate in old age and thought it could account for the decreased testosterone production rate. In 1968 Kirchner & Coffman (10), reported significantly lower plasma testosterone levels in a small group of elderly males age 55–65 (n = 6) in comparison to younger males age 18–38 (n = 12).

We ourselves (11) studied *plasma T* levels systematically in a group of 150 males, between 20 and 90 years old and observed a highly significant decrease in mean testosterone levels in each decade after age 60. However it became clear that this decrease did not apply to all individuals, as at age eighty for example, T levels might be either as low as in the female or as high as considered normal for a man age 30. Moreover we observed a highly significant increase with age in the binding capacity of the testosterone binding globulin (TeBG) : in our group of patients below 50 years of age (n = 30) the mean binding capacity was 3.5×10^{-8}M (95% conf. limits $2.9–4.1 \times 10^{-8}$M) whereas in a group of subjects over 50 years old (n = 27), mean binding capacity was 7.9×10^{-8}M (95% confidence limits $6.9–9.3 \times 10^{-8}$M), a highly significant difference ($P < 0.001$). This results in a highly significant decrease in the free testosterone fraction and concentration with age (table I). These findings were confirmed recently by Stearns et al (12). As expected we also observed an influence of age on dihydrotestosterone (DHT) levels : in males below age 50 mean DHT level was 66.6 ± 3.7 ng/100 ml (n = 47) against 51.9 ± 5.3 ng (n = 39) in patients over 50 yrs old ($P < 0.02$), the mean T/DHT ratio remaining constant [10.5 ± 0.8 in males < 50 yrs old (n = 37); 9.3 ± 1.3 (n = 14) in males over 65 yrs old.]

17-hydroxyprogesterone levels on the other hand which may be considered to be, at least under basal conditions, essentially of testicular origin were only slightly ($P < 0.05$) lower in males over 65 years old

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TABLE I—Testosterone Levels, Binding Capacity and Free Testosterone in Males as a Function of Age

	<50 yrs (n = 30)	>65 yrs (n = 30)	Stat. Sign. P
PLASMA TESTOSTERONE (ng/100 ml)	487* (407–583)**	264 (210–330)	<0.001
TeBG Capacity (10^{-8}M)	3.5 (2.9–4.1)	8.9 (6.9–9.3)	<0.001
FREE T Fraction (%)	2.5 (2.4–2.6)	1.2 (1.0–1.4)	<0.001
Apparent FREE T Concentration (ng/100 ml)	10.6 (9.3–12)	3.6 (3.1–4.3)	<0.001

*m
**(95% conf. limits of mean)

(mean 82 ± 11 ng/100 ml, n = 20) than in younger subjects (mean 112 ± 9 ng/100 ml, n = 35).

Surprising to us was the observation that the non-conjugated *dehydroepiandrosterone levels* decreased from a mean level of 470 ± 58 ng/100 ml (n = 24) in males under 50 yrs old to 175 ± 18 ng/100 ml (n = 28) in elderly males (P 0.001). As, in accordance with other authors, we showed DHEA to be essentially of adrenal origin, this would indicate a shift in adrenal cortical secretion with age.

Androstenedione on the other hand increased slightly but not significantly in old age, whereas *estradiol* levels were significantly higher in elderly males than in younger subjects (13) (table II). Besides decreasing the free testosterone fraction the increase in binding capacity of TeBG in old age has another consequence : due to lower binding affinity of estradiol for TeBG, at low binding capacity and at normal steroid levels the free testosterone fraction (in per cent) is higher than the free estradiol fraction, whereas at higher binding capacity the estradiol fraction becomes the more important; in (Fig. 1) we have plotted the free E2 and T fractions, determined by dialysis, as a function of TeBG capacity in a group of 50 males aged 20–90 years; the relative increase in free E2 fraction with respect to the free T fraction with increasing TeBG capacity is evident, and the curves obtained are in excellent agreement with the curves calculated by Burke & Anderson (14). As a result, although E2 levels in elderly males are only slightly (albeit statistically significantly) higher than in young males (13), the free concentration of estradiol is more significantly increased; moreover aging is characterized by a highly significant increase in the ratio of free estradiol to free testosterone (table 2).

TABLE II—Estradiol Concentration in Males as a Function of Age

Age	<50 yrs n = 30	>65 yrs n = 30
E_2 (ng/100ml)		
Geometric $\overline{m}$	1.4*	2.1*
±2 SEM	(1.1–1.7)	(1.7–2.6)
% FREE E_2		
Geometric $\overline{m}$	2.0	1.3**
±2 SEM	(1.9–2.1)	(1.2–2.4)
APP. FREE E_2 CONC. (pg/ml)		
Geometric $\overline{m}$	26	28
±2 SEM	(20–33)	(23–34)
$100 \times \frac{AFE_2C}{AFTC}$		
Geometric $\overline{m}$	0.23	0.98
±2 SEM	(0.18–0.29)	(0.79–1.21)

*$p < 0.02$
**$p < 0.001$

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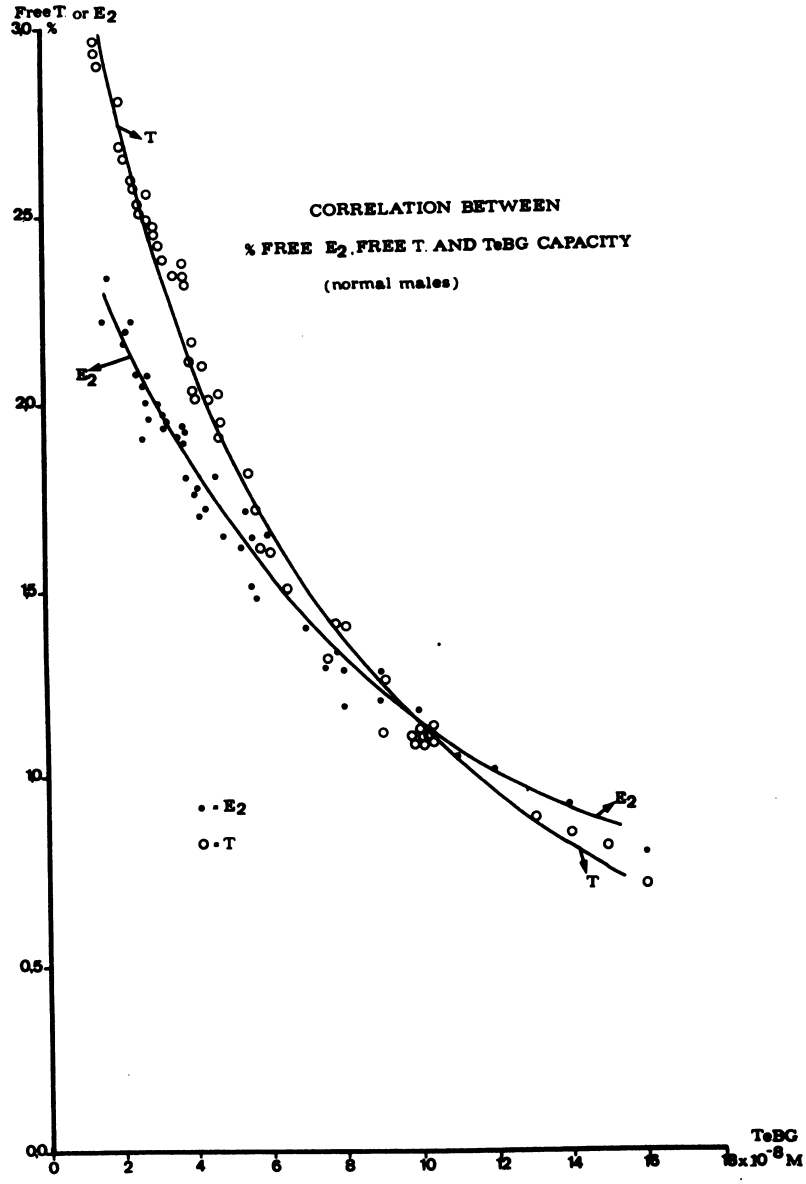

FIGURE 1. Free testosterone and free estradiol fraction respectively in a group of normal males age 20–90 (n = 50) as measured by equilibrium dialysis, plotted in function of the binding capacity of TeBG.

The increase in binding capacity of TeBG, with the concommitent decrease in free T fraction, is probably responsible for the decrease in metabolic clearance rate observed in the elderly : the decrease in T production rate therefore has a double origin : a decrease in plasma levels and a decreased MCR (table III). The next problem to investigate was the cause as well as the significance of this decrease in testosterone secretion.

The decrease in Leydig cell mass, as described by Tillinger (2) suggested that the decreased testosterone secretion has a primary testicular origin. In accordance with the groups of Winter & Faiman (12), as well as Schalch et al (14), Nieschlag et al (15) and Isurugi et al (16), we observed an increase of plasma LH & FSH levels in old age (Fig. 2).

Haug et al (7) as well as Wide et al (18) however did not observe an age related increase in LH levels. This increase in gonadotropin levels in old age confirms the persistence of an active feed back between gonadal hormone levels and LH secretion. Nevertheless, notwithstanding increased LH levels, the mean testosterone level remains below the mean normal level in younger adults, suggesting

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TABLE III—T Levels, T Metabolic Clearance Rate (MCR) and Blood Production Rate (BPR) as a Function of Age

Age	20–50 yrs n = 12	>65 yrs n = 12	Ratio Young/Old
Plasma T (ng/100 ml)	492 ± 50	380 ± 40	1.29
MCR (1/m²/24 hr)	640 ± 25	530 ± 35	1.21
BPR (mg/24 hr)	6.6 ± 0.5	4.0 ± 0.6	1.65

that the Leydig cells are not capable of increasing their secretion.

Therefore we studied the response of the Leydig cells to HCG stimulation. We (13), as well as Longcope (18), observed that upon HCG stimulation Leydig cells in elderly males increase their testosterone secretion and that, although the absolute increase is lower than in younger males, the relative increase is similar. These results ruled out a Leydig cell unresponsiveness as a cause of decreased T levels. Another possibility is an incapacity of the gonadotrophs to increase their LH secretion. In order to test this hypothesis we performed an LHRH stimulation test and compared the LH response in young and elderly males. We used a single dose I.V. stimulation with 200 μg of LHRH and observed that the levels of both LH and FSH reached were slightly higher in the elderly than in younger males; the relative increase was however somewhat lower (table IV).

Similar observations were made by Stearns et al (12), but our results were not confirmed by Haug et al (7) who observed a decreased pituitary response to 100 μg of LHRH.

These results indicate that in elderly males the gonadotrophs are capable of responding to hypothalamic LHRH stimulation with a further increase in LH and FSH levels. It appears, therefore,

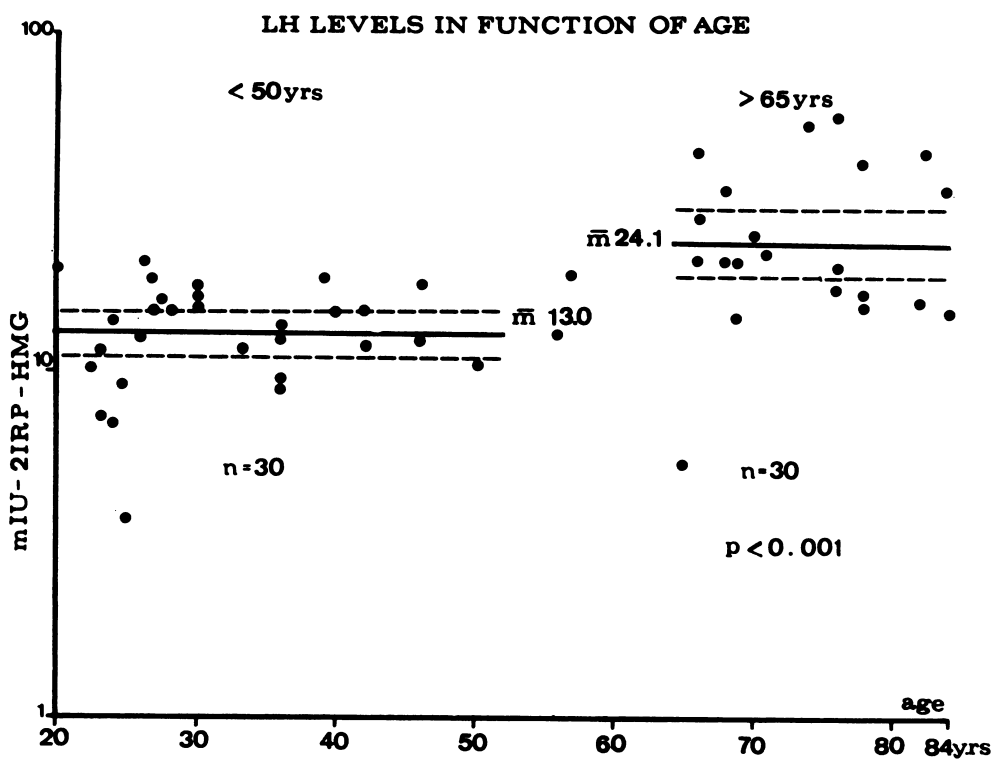

FIGURE 2. Plasma LH levels as a function of age (lograrithmic scale).

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TABLE IV—LH* Levels After 200 μg GnRH I.V.

	Basal value	20′	40′	60′	120′	ΣΔLH 20–60′
MALES <50 yrs						
geom.$\bar{m}$	13.0	62.5	69.1	56.9	33.8	165.1
(95% conf. limits)	(11.1–14.5)	(47.0–83.0)	(54.6–87.3)	(46.6–69.4)	(23.8–48.0)	(117–233)
In % of basal level		462	508	364	205	
(95% conf. limits)		(333–643)	(387–668)	(289–459)	(148–285)	
MALES >65 yrs						
geom.$\bar{m}$	22.5**	62.5	78.3	75.8**	67.3**	148.1
(95% conf. limits)	(15.9–31.8)	(46.8–83.3)	(60.4–94.6)	(62.0–98.6)	(51.7–87.7)	(110–199)
In % of basal value		278	337	335	258	
(95% conf. limits)		(220–385)	(244–468)	(244–461)	(302–329)	

*MIU/ml (LER 907)
** P <0.01

that both poles of the hypothalamo-pituitary gonadal axis are capable of increasing further their hormonal secretion. The modestly increased basal plasma gonadotropin levels in elderly males, notwithstanding decreased free testosterone levels, therefore suggest either a lowered set point of the testosterone feed back mechanism or that other factors besides free testosterone influence this feedback. As changes in plasma DHT levels grossly parallel those of testosterone, it is evident that this factor cannot be DHT. A possible candidate might be the free estradiol concentration. The latter is only slightly but not significantly increased in elderly males. As an approach to this problem we calculated the correlation between the LH levels and either testosterone, free testosterone, estradiol, or the free estradiol concentration. The best correlation ($r = -0.57$) was observed between LH and the free T fraction. The multiple correlation coefficient between LH and both free T and free E2 levels ($r = 0.44$, $n = 38$) was not significantly different from the correlation coefficient between LH and free testosterone. Hence, our results do not support the view that the increased free estradiol levels in elderly males might be responsible for the increased LH levels, notwithstanding low testosterone levels.

Summary

In summary, old age in males is characterized by a variably decreased Leydig cell function; as a consequence testosterone, dihydrotestosterone and 17-hydroxyprogesterone concentration are decreased. A concommitent increase in TeBG concentration results in a further decrease of the free testosterone concentration, and, for pure physicochemical reasons, in a relative increase of the ratio of free estradiol to free testosterone. The decreased Leydig cell function results in increased LH levels which, however, do not normalize the androgen levels. Since both the Leydig cells and gonadotrophs of elderly men react normally upon stimulation, it is tempting to postulate a lowered set point of the feed-back.

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DISCUSSION

S. G. KORENMAN: In the female the response to LRF is modulated by estrogen. This is true in intact humans and in animals as well and is a direct effect of estrogen on the pituitary. Have you correlated the response of estrogens in individual patients that were treated with LRF or looked at the correlation between serum estrogen levels and the response to LRF?

A. VERMEULEN: No, we have only calculated the means of the response and not tried to correlate individual response with any of the steroid levels in plasma.

O. HECHTER: You say that stress normally decreases testosterone levels. Is it possible in elderly men stress evokes increased secretion?

VERMEULEN: I don't think so; we have studied the effect of ACTH on plasma testosterone levels in elderly men and observed a significant decrease in plasma testosterone levels. We have also observed that heavy stress decreases plasma testosterone levels, and other studies, performed in this country, have also shown that physical stress decreases T levels.

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ANDROGEN HORMONES AND PREHORMONES IN YOUNG AND ELDERLY MEN

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Testicular Secretions in Young Men

The adult human testis secretes a number of steroids which have potential or actual biological activity. These substances include testosterone, 17α-hydroxyprogesterone, androstenediol, dehydroisoandrosterone, dihydrotestosterone, and estradiol. The major secretion product is testosterone, and spermatic vein concentrations range between 20 and 50 μg per cent versus 0.3–1.2 μg in peripheral blood (1–4). The product of testicular vein content and blood flow per unit time closely approximates the blood production rate of testosterone (5–10 mg per day), and this agreement and the very low plasma testosterone values in castrates (0.05 μg per cent) indicate that nearly all circulating testosterone arises from direct secretion.

The biological role of the other secreted testicular steroids is by no means clear. One and one-half mg of 17α hydroxyprogesterone is produced by the testis per day which is one third to one fourth that of testosterone (5). The amount of dehydroisoandrosterone (DHEA) secreted is very small, and the major fraction of blood DHEA as in females arises from the adrenal cortex. In contrast, another weak Δ^5, C19 androgen, androstenediol is produced in relatively large amounts (1–2 mg per day) since testicular vein blood content is about one fifth that of testosterone (6). The testis is now known to secrete small amounts of estradiol, and spermatic vein blood contains 100 ng versus 2 ng per cent in peripheral blood (7). The estimated direct testicular secretion is about 10 μg per day. In addition, the human testis is now known to secrete dihydrotestosterone (DHT), and spermatic vein plasma contains 0.6 μg per 100 ml versus 0.05 μg in peripheral plasma (8). These data suggest that the direct secretion by the male is 50—100 μg per day. Trace amounts of androstenedione, pregnenolone, progesterone, and 17α-hydroxypregnenolone, all biosynthetic precursors of testosterone, have also been found in spermatic vein blood (4).

Although a considerable number of testicular secretion products potentially play a hormonal role, probably only testosterone is secreted in large enough amounts in normal men to have a significant biological effect. Nevertheless, it is possible that some of these steroids do play some as yet undefined synergistic or individual role on target tissues. A number of these steroids (androstenedione, DHEA, estradiol, androstenediol, dihydrotestosterone) are not thought to be of great importance in the circulation because in blood they are derived primarily from the adrenal cortex (androstenedione, DHEA) or originate from peripheral conversion of other secretion products (estradiol, androstenediol, and DHT) (9).

Peripheral Conversion Products of Gonadal Steroids

It is now clear that certain hormones are derived from peripheral conversion of less active compounds. The term prehormone has been used to describe this phenomenon. Prehormones are endo-

Work supported by a grant from the National Institutes of Health (AM 13710).

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crine secretary products normally present in the body but that are converted peripherally to more active compounds and contribute to the overall biological effect. Peripheral conversion can occur in target tissues or at other sites (Fig. 1). The same active product may be secreted and also formed from other precursors (10). The first modern study of this relationship was performed by Lieberman and his group and Baulieu et al. from 1963–1965 with respect to dehydroisoandrosterone (DHEA) and its sulfate. Using isotopic techniques, these investigators proved that DHEA and its sulfate are interconvertible in the body (11). Horton and Tait subsequently determined that androstenedione in the female is converted peripherally to testosterone and is the source of two thirds of circulating testosterone (Fig. 2) (12). This situation also appears to exist in the prepubertal male where androstenedione production exceeds that of testosterone (10).

Recent studies have indicated that testosterone, although a potent androgen, is a precursor or prehormone for a number of other biologically active sex hormones. Secreted testosterone in man is converted into estradiol, androstanediol, and dihydrotestosterone (DHT) (Fig. 1).

When a constant infusion of a radiochemically pure precursor (prehormone) is given and a steady state is attained (infusion rate = disposal rate), a ratio of the concentration of radioactivity between the product and the precursor corrected for purification losses is obtained, and this is called the conversion ratio ($CR^{Pre\text{-}Prod}$). If the per cent of precursor infused that is converted to the product is measured in peripheral blood a conversion rate or transfer constant ($[\rho]^{Pre\text{-}Prod}$) is obtained. When the product of the precursor plasma concentration and the appropriate conversion ratio is calculated the amount of product in blood from precursor can be estimated. Similarly, the product of the precursor blood production rate and the transfer factor ($[\rho]$) yields the amount of product per unit time derived from the secreted precursor. When the overall production rate of the product is known, the percent of product from peripheral conversion and the remaining amount presumably from direct secretion can also be estimated (13). When quantitative studies of this type using labeled testosterone and estradiol are performed, testosterone appears to be a major prehormone for estradiol in the adult male (Fig. 3). In plasma 60–80 per cent of blood estradiol arises from peripheral conversion (14). The overall production rate of estradiol is 40–50μg per day, 30 μg of which is derived from peripheral conversion. A similar relationship exists between androstenedione and estrone in the male, but no biological consequence is known for estrone in men. It has

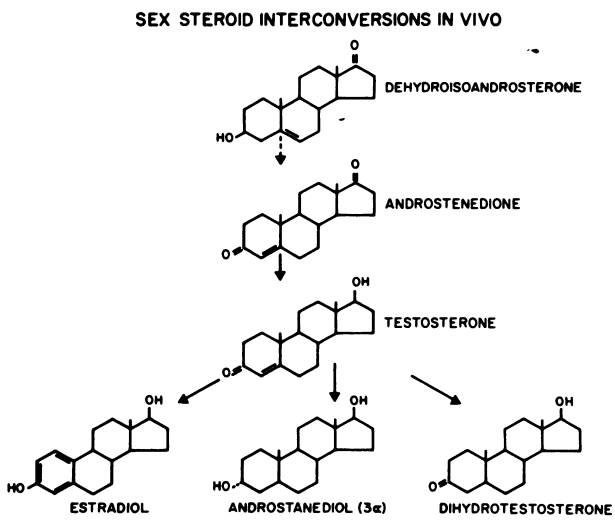

FIGURE 1

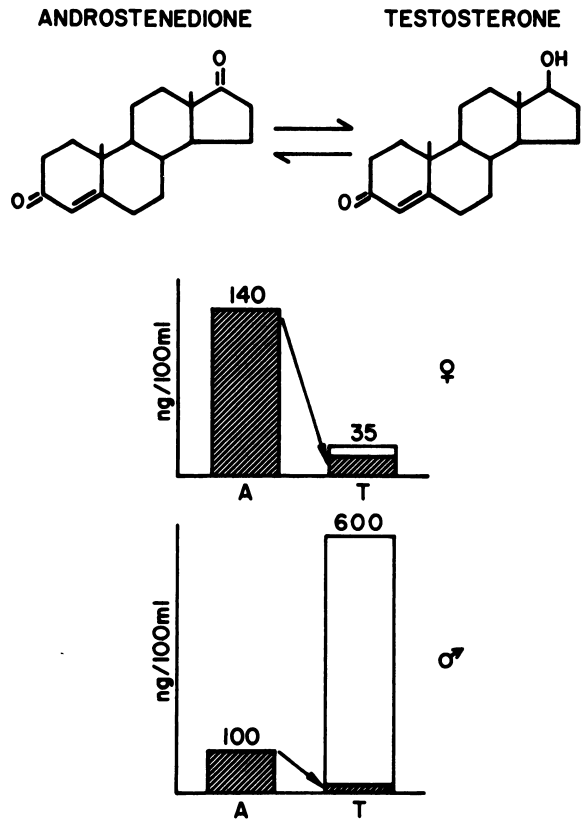

FIGURE 2

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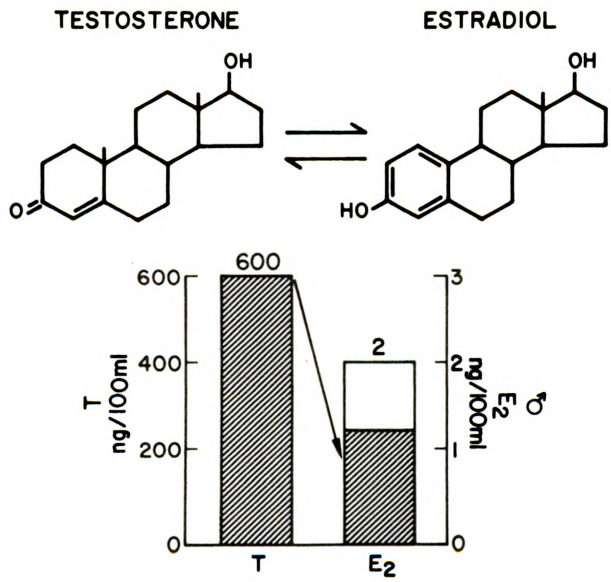

FIGURE 3

been suggested that testosterone feedback on follicle stimulating hormone is medicated by testosterone conversion to estradiol in the brain (15).

Androstanediol (5α-androstane-3α,17β-diol or 3α-diol) is a biologically active androgen with greater bioassayable activity than testosterone (16). 3α-diol can be metabolized in vitro to DHT, and perhaps this interconversion explains its biological activity since it is not bound to nuclear receptors from sexual target tissues. Testosterone is a major prehormone for blood 3α-diol (Fig. 4). Recently, a specific radio-immunoassay for plasma 3α-diol has been developed (17). There is a major sex difference in the circulating level of this androgen. Male values are 13.7±4 (SD) while adult female blood contains only 2±0.6 ng per 100 ml.

The blood production rate (PR_B) of a hormone can be approximated by the product of the mean plasma concentration and the metabolic clearance (volume from which complete and irreversible removal occurs per unit time on MCR) as follows:

$PR_B = i \times MCR$ where i is the mean plasma concentration. The blood production rate of androstanediol is 200±30 μg per day in men and 35±10 μg in women. An unusually high MCR was found suggesting that this steroid has unique metabolic and perhaps target cell characteristics. Kinetic studies by Mahoudeau and independently by our group indicate that 3α-diol in blood is derived almost entirely from its prehormone testosterone by peripheral conversion (18). Our studies indicate that >70% of 3α-diol is from testosterone conversion and that about 75% of this is derived via DHT (T→DHT→3αdiol). In contrast, in women only 10% of 3α-diol is from testosterone, and secreted DHT and androstenedione may be the prehormones (18). In men, physiological amounts of testosterone account for feedback regulation of luteinizing hormone secretion; the final brain mediator may, however, be DHT or 3α-diol as a result of peripheral conversion at the pituitary and/or hypothalamic levels (19).

Androstanediol-3β,17βdiol (3βdiol), an isomer of 3αdiol, is also derived from peripheral conversion of testosterone. It does not have the classical androgenic activity on nitrogen retention. However, it can cause prostate cell hypertrophy in tissue culture (20). Peripheral plasma levels of 3βdiol in both men and women are very low i.e. <2 ng per 100 ml (unpublished data).

Dihydrotestosterone (17β-hydroxy-5αandrostan-3-one) is 2–3 times more potent in most bioassays than testosterone (16). There has been considerable interest in this steroid both because of its high biological activity and due to the important work of J.D. Wilson's group indicating that testosterone is a prehormone in peripheral target tissue for DHT. Testosterone is converted into DHT by a 5α-reductase enzyme primarily located on target cell nuclear membranes. It is then transported into the nucleus and binds to specific nuclear chromatin where DHT acts like other steroid hormones at the level of nuclear transcription (21–23).

Testosterone itself, nevertheless, appears to

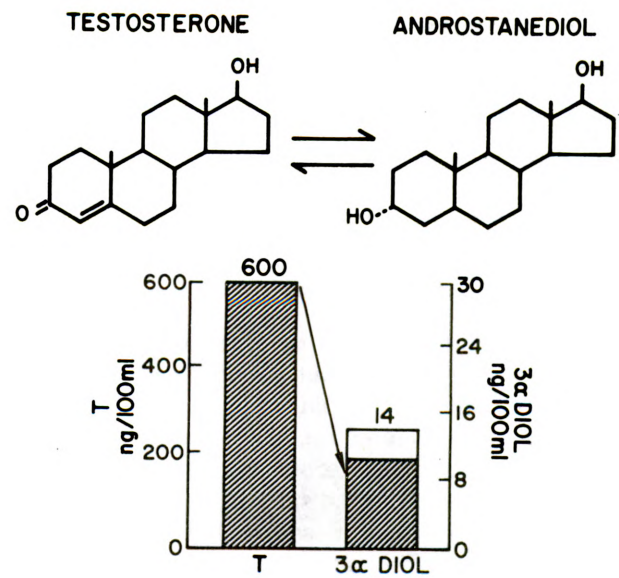

FIGURE 4

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mediate directly androgen actions as the anabolic messenger, ie. muscle development, and in Wolffian duct-epididymis-vas deferens-seminal vesicle development in the fetus. DHT appears to be the intracellular hormone responsible for development of the urogenital tubercle (penis) and the prostate (24). Wilson noted that after [^{3}H] testosterone injection into rats, small amounts of [^{3}H] DHT appeared in blood (25). We observed that DHT has a higher affinity for sex hormone binding globulin (SHBG or TBG) than testosterone (26) and subsequently developed a competitive protein binding assay for plasma DHT (27). Mean plasma DHT levels in mature young men are 54±19 ng per 100 ml (SD), prepubertal values are undetectable (< 10 ng percent), and concentrations in female blood are 15±5 ng. The normal testosterone/DHT ratio is, therefore, 600/50 or 12 in young men and 2.5 in women. A number of radioimmunoassays for plasma DHT have also been developed (28,29). Values obtained from young men have been 47±16 ng per 100 ml, in good agreement with the competitive protein binding method. Since there is now ample data suggesting that DHT synthesis occurs in peripheral target tissues, it was of interest to determine if blood DHT arises from secretion and/or peripheral conversion of secreted testosterone (Fig. 5). Using the constant infusion approach in young men, the MCR DHT was 650±35 (SD) liters per day in contrast to MCR^T (1000 liters per day), which was predicted on the basis of relative SHBG plasma protein binding affinity. The calculated blood production rates (i × MCR) were 300±65μg per day in men and 56±26μg per day in young women. The CR^{T-DHT} was also determined and was 5.6±0.6 percent in men. No back conversion to testosterone was detected as expected. The transfer constants ([p]) were 3.9±1% in the male and 1.7±0.6% in the female (30). Lipsett and coworkers and Ito, et al. both concluded from labeled interconversion studies that most of blood DHT is from secreted testosterone in men and from secreted androstenedione in women (31). Although the ratio between testosterone and DHT production is 20:1, the biological activity of DHT converts this chemical ratio to a biological ratio of perhaps 7:1. Therefore, DHT is a significant circulating androgen in the young adult male, and similar calculations indicate its even greater importance in female blood. Since secreted testosterone is the major prehormone for blood DHT and since in vitro studies demonstrated that sexual tissues are the major site of testosterone-DHT metabolism (32), it has been suggested that blood DHT production may be a

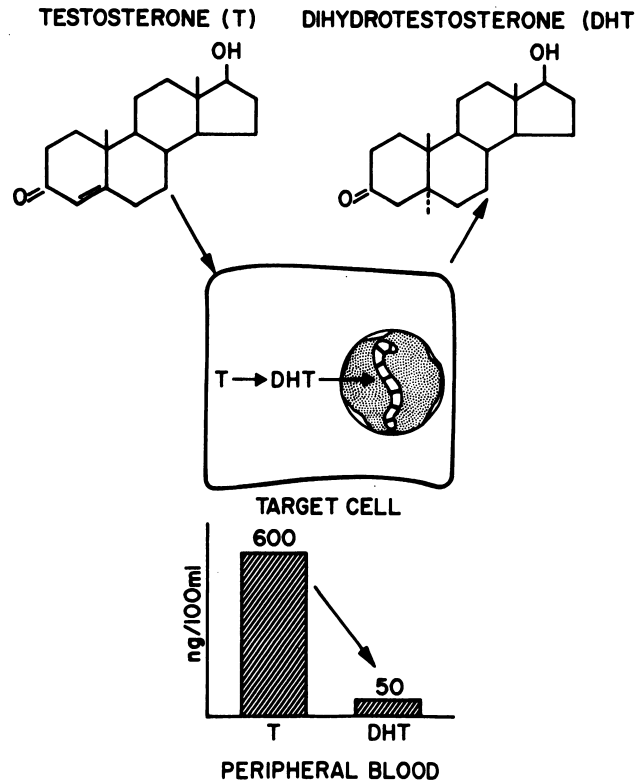

FIGURE 5

good reflection of target tissue events, ie. an in vivo reflection of target tissue events ordinarily only approached by in vitro techniques (30).

It is interesting that the kinetic interpretation suggesting that testosterone is a major prehormone for blood estradiol, androstanediol, and DHT have been confirmed by direct studies of testicular secretion rates. Analysis of testicular vein blood suggests that only approximately 25% of blood estradiol and 20% of DHT could be accounted for by direct testicular secretion. The agreement between such independent approaches lends great strength to these concepts.

Testosterone Production in Elderly Men.

It now appears that the aging testis secretes less testosterone particularly after age 60. This reduction in secretion rate has been verified by measurements in spermatic vein blood (32). The calculated blood production rate is further reduced in the elderly by a reduced metabolic clearance rate (33). Recently Vermeulen and coworkers studied plasma testosterone and apparent free plasma testosterone (dialysis of diluted plasma) in men ages 20–90. Mean

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testosterone values decreased progressively as did the free testosterone. Calculated blood production rates (i × MCR^T) were only 4±0.6 mg per day in elderly men versus 6.6±0.5 in young adults (34).

Dihydrotestosterone and Male Sexual Accessory Tissue.

It is now known that testosterone is specifically taken up by male accessory target tissue of the fetus and adult where it acts at the level of nuclear transcription. However, with respect to testosterone it appears that in peripheral target tissue, testosterone is a prehormone and is converted into dihydrotestosterone and other metabolites such as androstane - 3α,17β diol. These important findings have been adequately described in recent reviews of the subject (24, 25, 35).

Dihydrotestosterone: Studies in Vitro on the Prostate.

In a series of studies Wilson and his collaborators have extended their earlier observations that DHT is the final androgen messenger into a hypothesis that DHT may play a vital role in prostate growth and hypertrophy. There appears to be an excellent correlation between those species who develop prostate hyperplasia (BPH) and the relative rates of conversion of testosterone to DHT by prostate tissue.

This group has also reported a striking rise in DHT concentration with prostate size in man, and the highest DHT concentrations are found in periurethral tissues from BPH glands (36). Exogenous DHT appears to induce growth and hyperplasia of the dog prostate (37). However, a recent report from this group suggests that exogenous administration may not produce the same effect as intracellular production. These observations can be interpreted as suggesting an etiologic role for DHT and prostate hyperplasia (38).

Dihydrotestosterone and Androstanediol: Studies in vivo in elderly men.

The number of in vivo studies on circulating androgens and aging is quite limited. In the dog, Haltmeyer and Eik-Nes perfused the prostate with arterial blood via the prostatic arteries. They found that testosterone is a major precursor of DHT which was secreted by the prostate. In addition, they noted that the specific activity of DHT leaving the prostate was lower than infused testosterone suggesting that the prostate is able to synthesize DHT from other precursors i. e. that the dog prostate is an endocrine organ actively secreting DHT (39). When [^{3}H] testosterone and [^{3}H] DHT are given intravenously to men 30 minutes before prostatectomy, both hormones accumulated specifically in the prostate. After removal DHT was the major metabolite of T, whereas exogenous DHT was metabolized to androstanediols and androsterone (40). Mahoudeau has recently reported that in patients with BPH studied just before anesthesia for prostatectomy DHT levels in peripheral blood were normal; however, there was a higher content of DHT in prostatic than peripheral vein blood. No gradient in testosterone across the prostate was noted (41). We have recently studied circulating androgens in elderly patients with BPH. The patients studied were in reasonably good health on minimal medications who lived in nearby retirement communities. These patients (n=43) were ambulatory and found on examination to have 2–4+ prostate hyperplasia on rectal examination. Unfortunately our urologist felt all patients in our group, ages 65–85, had some degree of BPH, and no usable control elderly non-BPH patients were investigated at that time.

Plasma testosterone and DHT were measured by radioimmunoassay methods (42,27). The DHT values were verified by measuring the samples by two RIA techniques, one using paper chromatography and the other a celite column (29). Both techniques separate testosterone from DHT, and the values obtained by the two methods were not significantly different. In these elderly men, the peripheral blood values for DHT were significantly higher than those from young men ages 20–40, DHT 96±6 (SE) ng per 100 ml (range 50–200) versus 53±7 (SE) ng from young men (43). In contrast, plasma 3αdiol values were lower in a group of plasma samples from the elderly men. In these men with BPH, testosterone levels were lower than in young men, and therefore we find a major alteration in the peripheral testosterone/dihydrotestosterone ratio in elderly men with prostate hyperplasia. These studies may therefore complement the current in vitro work implicating either a cause or effect relationship between the androgen messenger DHT and prostate hypertrophy.

Speculations.

If our preliminary observations are correct, there is both an absolute and relative increase in blood

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DHT in contrast to testosterone in elderly men with BPH. What is the source of blood DHT in men with BPH? Possibilities include enhanced testicular secretion, altered conversion of prehormones (testosterone to blood DHT; by some unidentified tissue(s), and the possibility that the hyperplastic prostate is synthesizing and secreting increased DHT de novo. Studies to understand these possibilities are important, and it is also necessary for our initial observations to be verified by others. Men with BPH should be studied under non stressful conditions. Stress and anesthesia can alter gonadotropin production and periodicity in rats (44), and surgical stress reduces androgen levels in men (45,46). If the in vivo rate of conversion of testosterone to DHT is found to be increased in elderly men with BPH this will not be a unique observation; Hemsell et al. observed previously that in both men and women the transfer constant for the extent of conversion of plasma androstenedione to estrone ($[\rho]^{A\text{-}E_2}$) increases with advancing age (47).

A general hypothesis that testosterone exerts its action in some target tissues through its direct activity (anabolic) and in other target tissues via action as a prehormone appears tenable from both in vivo and in vitro studies. Further information is necessary in order to test more adequately whether conversion to estradiol or androstanediol is the neuroendocrine feedback signal and whether conversion to DHT is necessary for prostate growth and hypertrophy. Inhibition of DHT binding or action (48) may provide an investigative or even potential therapeutic tool. The possible relationship between events occurring in the hyperplastic prostate (BPH) and circulating androgen hormones and prehormones may be a new area of potentially valuable biomedical research.

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DISCUSSION

J. E. McNeal: I have two related questions about the cases of BPH. First, in regards to the men who had the prostatectomy, do you have information as to the relation between the testosterone values of each individual and the way the tissue was removed?

R. Horton: No, I have not made that correlation.

McNeal: Second, in the group of men with the wide variation of testosterone levels, were those people diagnosed as BPH mainly on the basis of urinary symptoms? In that case the scatter or var-

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iability in the weights of their prostate could be as variable as the testosterone levels that you showed, or were these men selected on the basis of how large their glands felt at palpation?

HORTON: Relatively healthy men were chosen, and they were evaluated by palpation by a urologist. They were selected by the presence or absence of symptoms. However, it turned out that nearly all men had some enlargement of the prostate. Therefore, no group with normal prostates was available to provide a true control group.

MCNEAL: But that could be quite variable in terms of their degree of BPH.

HORTON: Yes. Our aim was to study blood androgen in healthy, unstressed elderly men and to correlate androgen levels with age and if possible BPH. We are now working on a larger series to see whether the key factor is age or BPH and also to determine the source of DHT (direct secretion or peripheral conversion).

S. G. KORENMAN: Do you have a scattergram of the T versus DHT in 40 patients because it could be implied, at least in some of the groups with plasma testosterone and high plasma DHT that there was an increase in T to DHT conversion? You said earlier in the paper that 75% of DHT was derived from peripheral conversion of T and that variation in conversion would be interesting.

HORTON: The two went together. The higher T's were associated with higher DHT's, not lower testosterones being related to higher DHT's. We do have LH's in these and the mean LH is significantly increased.

KORENMAN: That would imply a large increase of conversion of T to DHT.

HORTON: Well, I think we are probably going to hear more of this from Dr. MacDonald today, but let me just make one point from some of the work from his group that may be analogous; he reported that there is an increase rate of conversion in vivo of androstenedione to estrone in both men and women with age, and we think its quite possible that this may be a more general phenomenon.

J. D. WILSON: That is complicated in regard to 5α-reductase, however, because at physiological levels the enzyme is not saturated. If you increase the substrate you also increase the amount of dihydrotestosterone formed because it is impossible to saturate the enzyme at physiological concentrations.

HORTON: The rate of delivery of testosterone to the periphery appears to be low by both overall production rates and free testosterone measurement.

WILSON: An interesting correlation would be the sum total of plasma T, DHT, and androstanediol in relation to age. Assuming that differences in clearance rate would be compensated in a steady state, it is conceivable that the net circulating level of all androgen might not change significantly with age.

HORTON: I believe that when T and DHT levels or production rate are combined that androgen production is quantitatively reduced. This concept supports the possibility that the prostate is more than just a target tissue and may be an endocrine organ secreting DHT.

J. T. GRAYHACK: Have you looked at the changes in DHT in men undergoing other major operative procedures?

HORTON: No, and I expected a question about the validity of the measurements of dihydrotestosterone. Dr. Vermeulen and I appear to have a discrepancy in our data, which is not at all that unusual in biomedical research, and it is possible that we may be dealing with separate populations. The stress factor should be considered and let me mention three references that suggest we should be very careful about evaluating the neuroendocrine gonadal system in patients. Dunn and coworkers reported in *Endocrinology* a few years ago that stress in rats alters both the basal level of LH as well as testosterone levels. It has also been observed in man by Uno and by Carstensen that stress, particularly anesthesia and surgery, lowers testosterone production.

VERMEULEN: I don't know about surgical stress except in patients undergoing prostatectomy in the next few days, but ACTH doesn't influence DHT levels.

HORTON: ACTH in some way lowers testosterone as was reported by Lloyd and co-workers quite a few years ago. Also, epenephrine lowers testosterone in males. These studies indicate that stress alters the male reproductive system.

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ORIGIN OF ESTROGEN IN MEN

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In men estrogen may originate from direct glandular secretion, i.e., testes or adrenal cortex, and from the aromatization of plasma C-19 steroid precursors, e.g., androstenedione and testosterone, at tissue sites other than the testes or adrenals. The sum of these two sources, i.e., glandular secretion and extraglandular production, makes up the total daily production rate of the two primary estrogenic hormones, estrone and estradiol. Quantification of estrone formation from plasma androstenedione and estradiol formation from plasma testosterone, as well as the total production rates of estrone and estradiol irrespective of their origin, have not been studied in aging men or in men with prostatic hypertrophy or prostatic carcinoma. Furthermore, while it is known that there is an increased efficiency of aromatization of plasma precursors with aging in men (1), the quantitative aspects of estrogen production in aging men are unknown. Although some controversy as to the origin of estrogens in men still exists, several groups utilizing varied approaches are in fundamental agreement regarding the origin of estrogen in young adult men (2–6). These studies suggest that estrogen production can be accounted for principally from the utilization of plasma precursors together with direct secretion of small amounts of estrogen, principally estradiol-17β, by the testis. Virtually all of estrone (E_1) is derived from one of two extraglandular sources. First, the conversion of plasma androstenedione to estrone accounts for 30 to 50 μg of estrone per day. Secondly, estrone is also derived from the peripheral conversion of estradiol (E_2) (which in turn was derived principally from the utilization of plasma testosterone). Little or no estrone is secreted directly either by the testes or by the adrenal cortex. Similarly, much of estradiol production can be accounted for from the utilization of plasma precursors. First, testosterone is converted directly to estradiol; and secondly estrone (derived from plasma androstenedione) is also metabolized in part via estradiol. These two extraglandular sources of estradiol, however, do not account for all of the estradiol produced in normal young adult men. On the average, 20–25% of daily estradiol production is due to testicular secretion. However, it should be emphasized that testicular secretion contributes only about 5–10 μg of estradiol production per day in normal young adult men.

In our studies, the entire daily production of estrone, measured from the specific activity of urinary estrone following the intravenous injection of [^{3}H]-E1, could be accounted for by extraglandular formation. In the case of estradiol, 1–9 μg/day of production could not be accounted for by extraglandular processes and is presumed to be due to testicular secretion. The mean testicular secretion of estradiol in our study was 6 μg/day.

Several investigators have quantified estrone, estradiol, and testosterone in spermatic vein plasma and compared these values with those found in peripheral venous plasma from the same subject. These data are summarized in Table I.

The T/E2 concentration ratios in spermatic vein were 686 and 586 in the studies of Kelch, et al. (4) and Weinstein, et al. (5), respectively. Baird, et al.

Supported, in part, by USPHS Grant AM 06912.

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TABLE I—Summary of reported comparisons of the levels of estrone (E1), estradiol (E2), and testosterone (T) in spermatic and peripheral venous blood.

Investigator	Spermatic Vein Steroid Concentration [mean, (pg/ml)]			Peripheral Vein Steroid Concentration [mean, (pg/ml)]		
	E1	E2	T	E1	E2	T
Kelch, et al. (4)	--	1049	712,000	--	20	5970
Weinstein, et al. (5)	306	1298	751,006	61	25	5600
Baird, et al. (7)	360	2081	--	74	32	--
Longcope, et al. (8)	99	342	59,000	39	32	4995

(7) did not measure T concentration. Longcope, et al. (8) collected "spermatic vein blood" through retrograde catherization through the renal vein, and these samples may have been diluted by nontesticular venous drainage. On the basis of the T-to-E2 concentration ratios and assuming a T secretion of 7 mg/day exclusively of testicular origin, Kelch and associates calculated the maximum mean testicular E2 secretion to be 10 μg/day. If one assumed a T secretion of only 4 mg/day, E2 secretion on the basis of data would have been only 5.8 μg/day. If T secretion were 2 mg/day, a likely value in Weinstein's subject #1 with peripheral T concentrations of only 2.8 ng/ml, the secretion of E2 in this subject (with spermatic T-to-E2 ratio of 872) would be 2.3 μg/day.

In the case of E1, the study of Weinstein, et al., is the most complete. The T-to-E1 concentration ratio in spermatic vein was 3042. Again, if we assume a testicular secretion of 7 mg/day of T, the computed secretion of E1 by the testis would be 2.3 μg/day. If the secretion of T were 4 mg/day, E1 secretion would be 1.3 μg/day. In Weinstein's case #1, the T-to-E1 ratio in spermatic vein blood was 12,929. Again, if we assume T secretion in this subject to be 2 mg/day, E1 secretion would have been 0.15 μg/day.

Therefore, we hold to our early conclusion: "In the normal [young] adult male, estrogen arises principally if not exclusively from plasma androstenedione and testosterone" (9). The conclusions of Kelch, et al. (4) and Weinstein, et al. (5) both working in Grumbach's laboratory, are similar to our own despite totally different experimental approaches. Other studies that have sought to measure plasma production rates of the estrogens may have overestimated the production rates for several reasons. First, the metabolic clearance rates from plasma of the estrogens may have been overestimated because the subjects were in a supine position during the infusions and because the infusion times were too brief to insure steady state conditions. Moreover, the concentration of plasma estrone may have been overestimated due to methodologic error and/or due to diurnal or episodic variation of estrone in plasma. That one or more of these alternatives affected the measurement seems probable since the reported plasma production rates of E1 exceeded those calculated from urinary data, a finding that is difficult to reconcile on physiologic grounds (10).

Similar studies have not been carried out in aging men. Considering the likely importance of alterations in androgen and estrogen production and metabolism in the genesis of prostatic hypertrophy, it is of importance that we quantify estrogen production in aging men and identify its origin; i.e., from extraglandular formation from plasma precursors or via direct glandular secretion.

Several studies have been carried out to quantify estrogen excretion into urine or estrogen concentration in plasma of older men (11). Moreover, Longcope (12) has studied the effect of HCG on plasma steroid levels in older men. Pirke and Doerr (11) did find that plasma estradiol increased in aging men. We have shown that there is an increase in the capability for extraglandular aromatization with aging in men. Of course, this finding must be related ultimately to the availability of the plasma precursors, androstenedione and testosterone, and compared with total estrogen production in the same individual. Previously, we have shown that orchiectomy in older men with prostatic carcinoma does not influence significantly the extent of aromatization of either plasma androstenedione or testosterone. Therefore, the testes of the older man do not utilize circulating androstenedione or testosterone as precursors of secreted estrogen. We also have shown that total prostatectomy does not influence significantly the extent of aromatization of plasma androstenedione or testosterone.

Summary

In the adult human male estrogen production

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arises from two sources. First, the principal source of estrogen is the extraglandular utilization of the plasma C–19 steroid precursors, androstenedione and testosterone. In young men estrone production is accounted for principally, if not exclusively, via extraglandular aromatization of androstenedione plus the conversion of testosterone to estrone by the sequence testosterone→estradiol→estrone. Estradiol arises by the extraglandular conversion of testosterone to estradiol and indirectly through the sequence, androstenedione→estrone→estradiol. In addition, there is a small amount of estradiol secreted by the testes, this amounting to approximately 5–10 μg/day. The origin of estrogen in elderly men has not been definitively determined. However it has been ascertained that the extent of extraglandular aromatization of the plasma C–19 steroids increases with age and body weight in men and women.

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DISCUSSION

O. HECHTER: Why is there an increased conversion with age?

P. C. MACDONALD: In part, it may be related to the decrease in liver function that has been documented with aging. I would rather wish to believe that ultimately it is related to the 5α-reductase system and there is a derepression of aromatase activity as a result of aging. It is interesting, as Dr. Moore stated yesterday, that the most potent inhibitor of the aromatase system is 5α-androstanedione or androstanediol.

R. M. BOYAR: Have you looked at patients with hypothyroidism? There seems to be a decrease in the deiodination of T_4 to T_3 with aging and lower serum T_3 levels.

MACDONALD: No. But Southren found an increase in peripheral conversion of androgens to estrogen in hyperthyroidism.

HECHTER: Do you have any idea besides adipose tissue where aromatization takes place in the body?

MACDONALD: As Dr. Horton mentioned, Ryan and coworkers have shown that it occurs in the hypothalamus. Dr. Wilson and coworkers have also shown that it occurs in the skin fibroblasts and in hair follicles. However, I think the bulk of it, in terms of daily conversions, takes place in adipose tissue.

G. WILLIAMS-ASHMAN: Is there any possibility that bacteria in the gut or skin could be involved in the steroid conversions you have reported?

MACDONALD: Well, we were concerned about this some years ago and performed studies following the administration of neomycin. We also collected all of the T-tube drainage following common duct exploration and that did not affect the overall extent of conversion so that in terms of conversion to estrone bacterial action is not likely. Whether or not bacteria might affect the formation of estriol is not clear.

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TESTIS: CHANGES WITH AGE

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Introduction

Morphological and functional changes occur in both tubular and interstitial elements of the human testis during neonatal life, again at puberty and finally during senescence (1,2). Herein the author focuses upon the testicular consequences of senescence in the human male. Where illuminating, information derived from experimental animals is included.

In contrast to the human female, changes in gonadal activity during male senescence are slow rather than climacteric. Moreover, the time of onset and rate of senescent changes in testicular function vary widely between individuals within a species and even between different anatomical locations within the testis. The effect of disease and nutrition are frequently confounded with senescence making it difficult to elucidate specific effects of advancing age upon testicular function.

General

Outward signs of diminished testicular function include reduced numbers of orgasms (3) and an increased incidence of impotence (4) in the human male as well as diminished fertility in mice and bulls (4,5,6,7,8). Unfortunately, these are only indirect measures of testicular endocrine and spermatogenic function and might therefore only represent the debilitating effects of old age upon the animal; consequently they do not constitute direct proof that advancing age results in specific testicular lesions.

Seminiferous Tubules

Spermatozoa are the principal product of the seminiferous tubules. Incontrovertible proof that senescence results in deleterious changes in spermatozoa is seen from the elegant studies showing decreased fertility of bovine spermatozoa obtained from bulls of advancing age in artificial insemination centers (6,7,8). This decline in sperm fertilizing capacity with advancing age is probably real since disease, nutrition and environment were controlled, since standardized semen collection and storage techniques were used throughout the experiment and since equivalent numbers of viable, motile spermatozoa were artificially inseminated into each recipient female. Unfortunately, this effect of aging upon spermatozoan fertility does not prove the existence of some primary seminiferous tubule defect since diminished spermatozoan fertility might also result from senescent changes in the epididymis or in seminal plasma. Consequently, we must next examine the seminiferous tubule directly.

Numerous investigators (2,9,10,11) have reported age related morphological changes within the seminiferous tubule of the human testis. Most of

Supported in part by NICHD Research Contract No. 3-2745, Research Grant No. 07204, Training Grant No. 00109 and Population Center Grant No. 06268.

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these investigators would agree that there are few senescent changes in the Sertoli cell but that hypospermatogenesis and collagen fiber infiltration with subsequent thickening and eventual hyalinization of the basement membrane occur during senescence. Disagreement centers about the time of onset and rate of progress of such morphological changes, whether or not one testicular region is more prone towards these senescent changes than another and whether these changes are associated with alterations in testicular vasculature.

Cautioning that there are notable exceptions, most authors would agree with Sasano and Ichijo (12) who concluded that the mean number of seminferous tubules containing spermatids is around 90% during the third and fourth decades, drops to 50% in the fifth decade, and finally approaches 10% in the ninth decade of life. Sasano and Ichijo (12) carried their analysis one step further when they showed that "these senile testicular changes were not uniform in severity throughout the testis but instead were induced in a pattern closely related to testicular blood supply". This observation, although not universally accepted (13), is worthy of detailed discussion.

According to Sasano and Ichijo (12), "the main arteries of the testis in Japanese males penetrate the capsula albuginea adjacent to the hilus and descend along the medial surface of the testis. The main arteries then turn up along the lateral surface to the upper pole and spread several branches along the seminiferous tubules toward the mediastinum". In other words, Sasano and Ichijo propose that "the lower and anterior parts are proximal to the arterial trunk and the upper part and mediastinum are situated at the periphery of the testicular blood supply". Sasano and Ichijo (12) then measured the percent of tubules showing spermatogenesis in six different horizontal and three different saggital sections of 121 human autopsy cases ranging from 14 to 73 years of age. Histological inspection of these sites in the human testis revealed that among the six horizontal sections, the anterior part consistently had the highest percentage of seminiferous tubules showing spermatogenesis while the posterior part, at the periphery of arterial supply, had the lowest in all decades of life. Moreover, as expected from vascular distribution, spermatogenesis was more depressed with advancing age in the upper of the three saggital testicular sections situated at the periphery of arterial supply. Provided Sasano and Ichijo are correct, their observations suggest an interesting explanation of the variability in time and rate of onset of seminiferous tubular lesions in human testes. Unfortunately, these results are qualitative and do not provide a quantitative estimate of the effect of senescence upon the actual production of spermatozoa.

Ewing *et al.* (14) estimated the total daily sperm production in aging rabbits by determining the number of spermatids and spermatozoa in testicular homogenates. Importantly, this technique allowed the determination of potential sperm production of rabbits, thus circumventing the confounding effects of reduced libido or increased epididymal sperm resorption, which might confound results obtained with ejaculated spermatozoa in rabbits of different ages. The aging rabbits were housed in air conditioned, light controlled quarters and only those animals maintaining or gaining body weight and in apparent good health were tested. Results in Fig. 1 show that sperm numbers per gram of testis increased through two years, then declined significantly ($P < 0.01$) between two and three years of age. Total daily sperm production was calculated by multiplying sperm concentration by paired testes weight and dividing by the appropriate time divisor derived from the length of one spermatogenic cycle (15). Analysis of variance showed that daily sperm production in rabbits increased through two years but decreased significantly ($P < 0.05$) by three years. That this aging effect on sperm production translated into fewer cells available for ejaculation is seen in the significant ($P < 0.05$) reduction of sperm cell numbers in the cauda epididymis between one and three years of age. Thus it appears that senescence causes changes in the seminiferous tubule that ultimately lead to decreased numbers of fertile spermatozoa available for ejaculation. Interestingly, it appears that different regions of the human testis may be more or less prone to these senescent seminiferous tubular changes.

Leydig Cell

A decrease in libido (4 and 15–17), number of orgasms (3), seminal plasma fructose (18,19) and spermatogenesis (9–12 and 14) in senescent males suggests that Leydig cell production of androgens declines with advancing age. Since these symptoms could also be explained by increased testosterone metabolic clearance or refractoriness of peripheral target organs to testicular androgens, we must next focus on specific Leydig cell changes with age.

Lynch and Scott (20), using light microscopy, estimated the lipid content of Leydig and Sertoli cells in 168 human testes stained with oil-red-O. The patients from which the testes were derived

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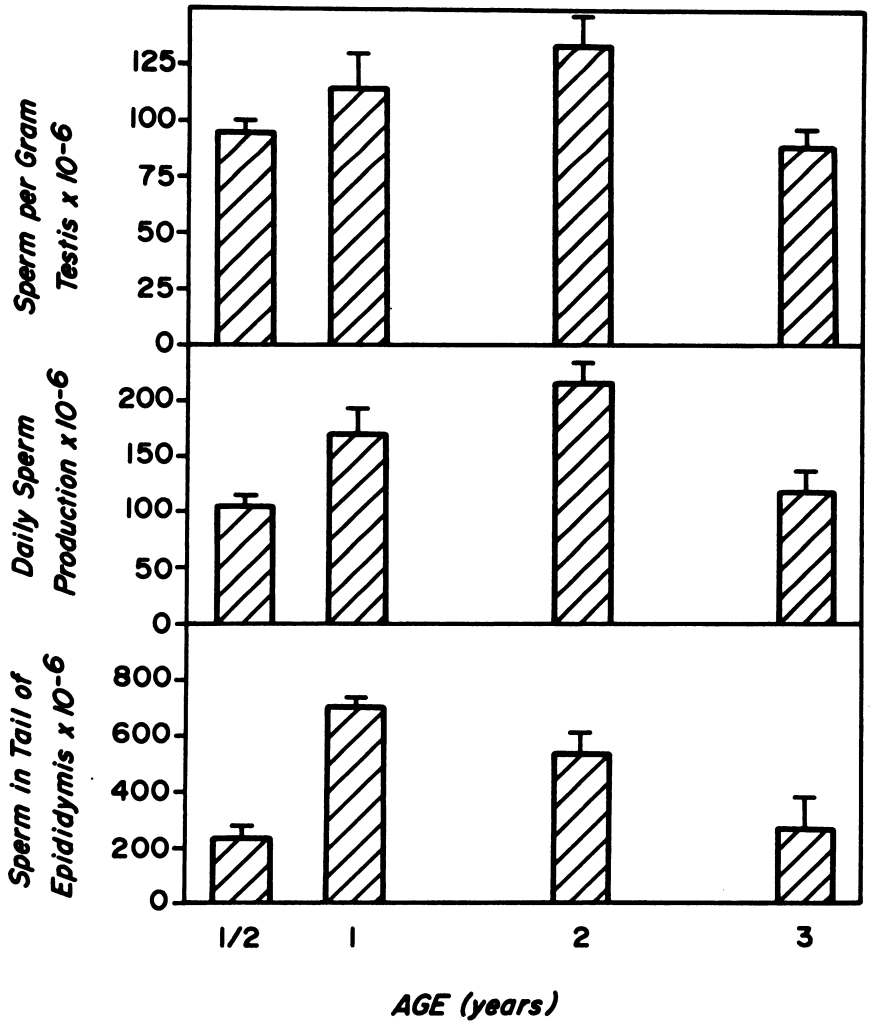

FIGURE 1. The effect of aging on spermatid and spermatozoa numbers (10^6) present in rabbit testes and epididymides. Top panel shows sperm per gram testis; middle panel shows daily sperm production; bottom panel shows sperm in one cauda epididymis. Each bar represents the mean ± standard error of the mean for 10, 10, 10 and 7 animals at 1/2, 1, 2 and 3 years of age respectively.

ranged from 2 months premature to 84 years of age. Leydig cell lipid appeared at 12 years of age, increased to a peak by 17 years where it remained until 35 years and then diminished gradually thereafter. Although qualitative in nature this elegant study suggested striking changes in Leydig cell function of human testes with advancing age. Attempts to estimate quantitatively the effects of senescence upon Leydig cell numbers have led to conflicting results and considerable controversy (9,21,22,23). Support for Lynch and Scott's observations (20) and resolution of the conflict about aging and testicular Leydig cell numbers come most recently from Harbitz's (24) careful and thorough morphometric study of Leydig cells in elderly men. Harbitz (24) used a statistically valid, precise and accurate technique to estimate the acutal weight in grams of Leydig cells in paired testes from 172 patients ranging from 40 to 80 plus years of age. As might be expected from Lynch and Scott's cytochemical observations (20), the mean Leydig cell weight declined gradually after 50 years of age. Importantly, the mean correlation between Leydig cell weight and age in men over 40 years was statistically significant ($P < 0.01$). Although illuminating, Lynch and Scott's (20) results are qualitative while Harbitz's (24) measurement of Leydig cell numbers is at best only an indirect assessment of Leydig cell function.

Evidence that the actual production of testosterone was reduced with advancing age in human males derived from studies where the 24 hour urinary excretion of testosterone was measured. Predictably, investigators (25,26,27) found that urinary testosterone excretion declined with advancing age. However, once again these experiments were

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open to more than one interpretation. For example, it is possible that urinary excretion of testosterone might not reflect decreased testicular production but instead only decreased amounts of free plasma testosterone available for catabolism and ultimate excretion. That this cautious interpretation was justified is seen in the recent evidence showing that aged males have higher concentrations of plasma testosterone binding globulin (28) and that this results in less free testosterone in peripheral plasma (28,29,30,31), thus reducing the free plasma testosterone which appears to be more readily available for metabolism and excretion (28,32,33) than protein bound testosterone. However, additional evidence that testosterone production declined during senescense was derived from measuring blood production rates. Plasma testosterone concentration, metabolic clearance rate and thus total 24 hour testosterone production all decline with advancing age in the human male (28,34,35).

Ultimate proof of a direct effect of aging upon testicular production of testosterone was derived from the direct measurement of testosterone concentration in spermatic vein blood of human males varying from 30 to 78 years of age (36). The level of testosterone in human spermatic vein blood varied from 1.6 to 0.025 μg/ml and decreased with advancing age. Unfortunately, no mention was made of testicular blood flow and thus it was impossible to state categorically that testosterone secretion was diminished in the testes of the aging men studied.

Ewing and co-workers (14) measured testosterone secretion with advancing age in rabbit testes perfused in vitro with an artificial medium (Fig. 2). Testes were perfused for 2 hours without exogenous gonadotrophin and 4 hours with artificial medium containing 4.0 μg FSH and 8.0 μg ICSH/ml. This concentration of gonadotrophin was used because it resulted in maximum testosterone secretion by the perfused testis. The amount of testosterone secreted during the first two hours did not differ significantly with age in the absence of exogenous gonadotrophin (Fig. 2). In contrast, testosterone secretion during the fifth and sixth hours of perfusion diminished from 0.83 to 0.45 μg/hr in testes from ½ and 3 year old rabbits, respectively (Fig. 2). Analysis of variance showed that there was no significant difference ($P < 0.15$) between the rate of testosterone secretion in testes of ½, 1 and 2 year old rabbits but there was a significant difference ($P > 0.05$) between testes of ½ and 3 year old rabbits. Thus these data conclusively prove that testes from 3 year old rabbits failed to secrete as much testosterone as testes from ½ year old rabbits when perfused in vitro with saturating concentrations of exogenous gonadotrophin.

The in vitro perfused rabbit testis presents a unique opportunity to study the effect of aging upon the responsiveness of Leydig cells to exogenous gonadotrophin since the artificial medium contains only minimal amounts of gonadotrophic hormones. Results of several experiments (14,37,38) have shown that perfusion of rabbit testis for 2 hours with artificial medium reduces testosterone secretion to a low basal level. These same experiments (14,37,38) have provided data showing that addition of saturating concentrations of exogenous gonadotrophin to the artificial medium maximally stimulated testosterone secretion within three hours of in vitro perfusion. Consequently, it was a simple matter to test the effect of aging upon Leydig cell responsiveness to exogenous gonadotrophin by perfusing testes from each age group with artificial medium for two hours in the absence, and for four hours, in the presence of saturating gonadotrophin concentrations. Predictably, results in Fig. 3 show that testosterone secretion of all age groups of animals increased significantly ($P < 0.01$) above basal levels of secretion upon addition of gonadotrophic hormones. Interestingly, the response to identical gonadotrophin stimulation declined significantly ($P < 0.05$) with advancing age. That this may, in fact, occur in the human testis is seen from several studies (39,40) showing a small but apparently real diminution in plasma testosterone response to identical dosages of gonadotrophin with advancing age. These data, coupled with the fact that the pituitary of aged men (40) respond similarly to those of young men when challenged with identical dosages of LHRH, suggest that the Leydig cell, and not the pituitary, represents the primary defect causing reduced testosterone secretion in senescent males.

At this juncture, it is of interest to note the temporal relationship between testosterone secretion by the perfused rabbit testis and daily sperm production in the same group of aging rabbits since testosterone is required for complete spermatogenesis in several species including rabbits (38,40). Results in Fig. 3 show that sperm production increased through 2 years of age proving that testicular testosterone production was adequate to maintain spermatogenesis. The significant ($P < 0.05$) decrease in daily sperm production coincident with the significant ($P < 0.05$) decline in testosterone production by perfused testes of 3 year old rabbits is suggestive of a cause effect relationship.

Unfortunately, there is little or no information available as to why Leydig cells become less re-

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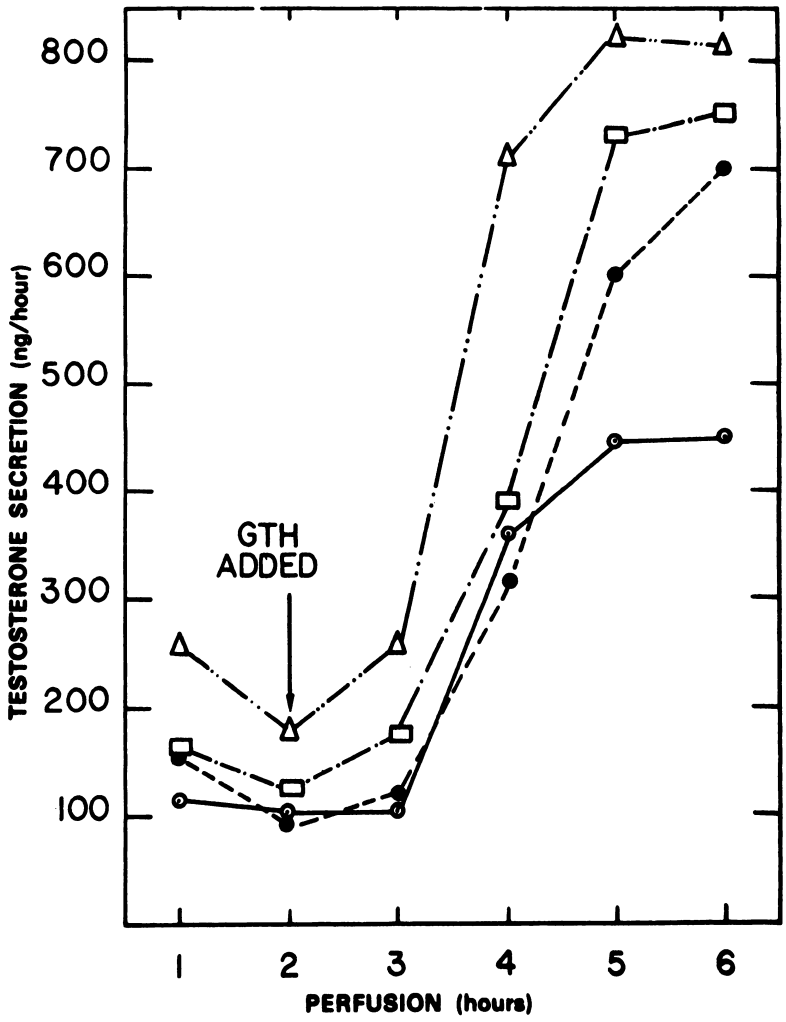

FIGURE 2. Testosterone secretion by perfused rabbit testes. (△-..) 1/2 year old rabbits (n=9); (□-.) 1 year old rabbits (n=7) .-2 year old rabbits (n=9); and ●· -) 3 year old rabbits (n=7). By courtesy of the editor of the Proceedings of the Society for Experimental Biology and Medicine.

sponsive to gonadotrophins with advancing age. The possibilities include death and subsequent loss of Leydig cells, impairment in the membrane receptor, disruption in the second messenger system (e.g. adenyl cylase) or finally breakdown of the steroid biosynthetic machinery itself. Unfortunately, few studies have been designed to investigate these eventualities. This reviewer found no studies investigating the effect of senescence upon Leydig cell gonadotrophin receptors or upon the second messenger system. Only two reports were found testing the effect of advancing age upon the testicular steroid biosynthetic machinery.

Kobayashi and Ichii (42) tested the effect of aging upon the activity of cholesterol side-chain cleavage in rat testis. These investigators found that the level of cholesterol side-chain cleavage in old rats was similar to that of young rats even though histological examination revealed little spermatogenic activity in testicular tissue of aged rats. These data support the arguments that the availability of pregnenolone for androgen biosynthesis is almost constant between young, mature, and aged rats.

Axelrod (43) measured the synthesis of testosterone intermediates in testes from one young, and one aged man. Although hardly conclusive because of the small number of testes used these results showed that 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, and 20α-hydroxy-dehydrogenation were similar in the young and aged testis. In contrast, the 17–20 lyase enzyme activity was greatly reduced in the aged tissue suggesting some effect of senescence upon this specific enzyme. Unfortunately, this latter finding has been neither ex-

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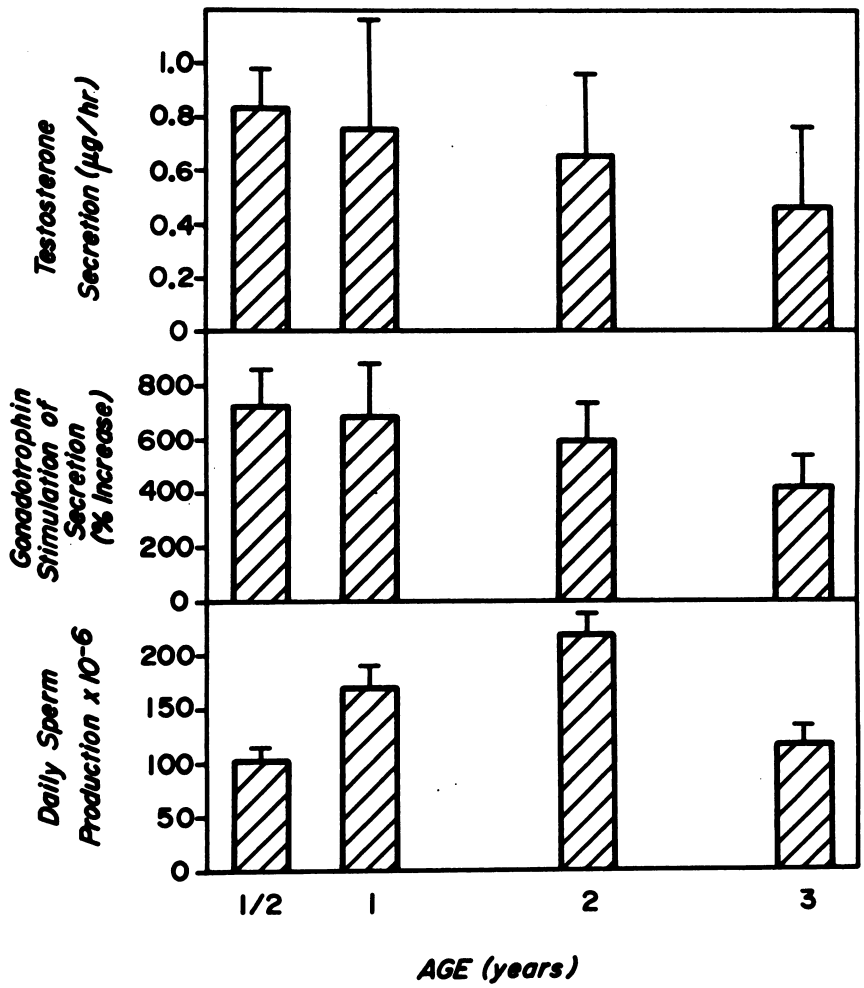

FIGURE 3. The effect of aging on testosterone secretion and daily sperm production in rabbits. Top panel shows the average testosterone secretion during the fifth and sixth hours of *in vitro* perfusion; middle panel shows the percentage increase in testosterone secretion (second hour versus average of fifth and sixth hour) in response to addition of 4 µg/ml follicle stimulating hormone (ovine, NIH-FSH-S7) and 8 µg/ml luteinizing hormone (ovine, NIH-LH-S16). Bottom panel shows daily sperm production. Each bar in top and middle panels represents the mean ± standard error of the mean for 9, 7, 9 and 7 animals at 1/2, 1, 2 and 3 years of age respectively. Each bar in the bottom panel represents the mean ± standard error of the mean for 10, 10, 10 and 7 animals at 1/2, 1,2 and 3 years of age respectively.

panded by Axelrod nor confirmed in other laboratories.

Discussion

It is clear that advancing age in the human male as well as in other species is accompanied by striking changes in testicular function manifested in reduced numbers of orgasms, decreased fertility and increased incidence of impotence. It is also clear that these outward manifestations merely reflect highly variable yet inexorable deleterious changes in spermatogenic and steroidogenic functions within the testis.

The decline in spermatogenic function during senescence is characterized by an extremely variable time of onset and rate of morphological change in the seminiferous tubule with ever diminishing numbers of tubules showing complete spermatogenesis, thickening of the basement membrane of the seminiferous tubule, subsequent hyalinization of the tubule basement membrane, and ultimately almost total loss of spermatogenesis. Interestingly, the Sertoli cell appears to be relatively immune to the ravages of advancing age. Some anatomical compartments of the human testis may be affected by senescence at an earlier age than other sections. Those compartments of the testis sensitive to aging may be poorly vascularized. Correlative evidence in male rabbits

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suggests that the decline in daily sperm production with advancing age is coincident with the significant reduction in testosterone secretion by testes from the same animals. Considerable work remains to be done, however, to prove whether a cause effect relationship exists between the senescent changes in testosterone secretion and spermatogenesis.

Leydig cell numbers and lipid content of Leydig cells in the human testis decline with advancing age. That these changes probably reflect reduced steroidogenic function is seen in that testosterone urinary excretion rate and 24 hour testosterone production decline with advancing age. Direct proof that testicular testosterone production declines with age was obtained by measuring the concentration of testosterone in spermatic vein blood of the human male and by measuring testosterone secretion by rabbit testes perfused in vitro. Not only do these data prove that aged testes produce less testosterone than young testes but the in vitro perfusion results suggest that either Leydig cell numbers decline with advancing age or they become refractory to gonadotrophic hormones. Unfortunately, very little evidence is available to pinpoint the mechanism by which aging reduces testicular testosterone secretion.

Several questions probing the effect of aging upon testicular function must be answered before we can understand fully the effects of senescence upon testicular function. Is the decline of spermatogenic activity in the senescent testis dependent upon or independent of the decline in testosterone production? Which spermatogenic cell types are first lost during senescence? What is the effect of advancing age upon Sertoli cell morphology and function? Several questions come to mind in this regard: Are Sertoli-Sertoli tight junctions and thus the blood testis barrier maintained during senescence? Does the FSH binding capacity of Sertoli cells change with advancing age? Is there any alteration in the production of androgen binding protein with advancing age? What is the chemical nature of the lipid accumulating in Sertoli cells of the aging testis? The mechanism by which advancing age inhibits testosterone production must be investigated. This suggests several important questions. Does the number or avidity of Leydig cell LH receptors change with age? Does the amount or physical-chemical characteristics of Leydig cell adenyl cyclase change with age? Does the activity of any or all of the enzymes on the biosynthetic pathway to testosterone change during senescence? Other unrelated questions which should be addressed include: what estrogenic steroids are secreted by the human testis? Does the quantity or type of testicular estrogen secretion change during senescence?

Summary

Aging in the human male is accompanied by striking changes in testicular spermatogenic and steroidogenic function. In contrast to the human female, changes in gonadal activity during male senescence are slow rather than climacteric. This coupled with the frank variation in the time of onset and rate of these changes in the human testis make it difficult to elucidate the mechanism acting to bring about diminished testicular function during senescence. Although not universally accepted it appears that different anatomical regions of the human testis may be more or less prone to senescent seminiferous tubular changes. The decline in testosterone production accompanying senescence in the human male seems to reflect a primary defect in the Leydig cell rather than the pituitary gland. The mechanism underlying this Leydig cell defect with advancing age is only poorly understood at this point in time. Moreover, it is unknown whether the decline in testicular testosterone secretion is causally related to the decline in spermatogenesis with advancing age.

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DISCUSSION

G. WILLIAMS-ASHMAN: I was interested in the pictures that you showed concerning different degrees of spermatogenesis in different parts of the testes of elderly people. Is it known whether the temperature of the testes in their normal scrotal position may vary from one part of the testes to another? Also, is there any evidence that intratesticular temperatures vary with age?

L. EWING: As far as I know, the answer to both of those questions is no, but Sasano and Ichijo have looked at the vascular supply of the human testis. There is usually a single artery that descends into the testis and runs along the posterior aspect of the gland. The artery penetrates the tunica vaginalis and gives off a series of branches that run over the periphery of the testis before entering the parenchyma of the tissue. Moreover, the vasculature tends to be concentrated along the lateral aspect of the testis. This probably provides additional cooling of the blood in the spermatic artery prior to its entry into the parenchyma. I would guess that there might well be local differences in testicular temperature.

R. F. GITTES: Pursuing the observation about the difference in sperm production in the parts of the testes, differences in temperature would probably not be as significant as differences in the distribution of Leydig cells. We have come to accept the fact that the spermatogenesis depends on the local diffusion gradient or concentration of testosterone. The testis may be irregular in the distribution of its interstitial cells.

EWING: I do not know of any evidence suggesting differential distribution of Leydig cells.

J. A. THOMAS: Several years ago I did some studies measuring intertesticular temperatures in rats at room temperature, and there was no more than a half to a degree variation. We had three needles inserted with approximately the same pole positions as you indicated schematically, and the mean temperature was approximately 30°C. So, temperature variation probably ought not be a consideration, but what about the cell cycles? I think perhaps it is more important than temperature. How does one synchronize cell cycles?

EWING: I wish I knew.

R. HORTON: To continue the discussion about why the Leydig cells and seminiferous tubules do not respond normally to stimuli in the elderly, there are many examples of reduced tissue responsiveness as part of the aging process. In endocrinology the aging thyroid does not respond normally to TSH. I think one of the approaches is to consider this as one of the phenomena of aging, and what attracts me about the prostate is that this may be a tissue which has a unique response.

J. D. WILSON: Does gonadotropin regulate the production of testosterone per cell or the number of cells or both?

EWING: The acute effects of gonadotropin upon Leydig cell steroidogenic activities almost certainly can be explained by increasing the production in each cell. Chronic effects of gonadotropin upon Leydig cell steroidogenesis are difficult to assess.

WILSON: Is the number of Leydig cells in the testes fixed at an early age?

EWING: Dr. Vermeulen showed data in which the Leydig cell numbers appear to decline with advancing age. Dr. Harbitz (Acta. Path. Microb. Scand., Section A, 81:301–314, 1973) published a very careful morphometric analysis of Leydig cell numbers in young and aged men in which he also showed that the mean Leydig cell mass declined after 50 years of age in men.

O. HECHTER: As I listened to these last papers documenting the steady decline with aging in certain kinds of androgenic components together with a protective mechanism for maintaining estrogens, the question that comes to mind is whether the treatment for benign prostatic hypertrophy may not be to give testosterone?

WILSON: There were clinical trials with testosterone, but the difficulty with those experiments is that you also increase the substrate for peripheral aromatization.

O. A. HAUGEN: I would like to mention data from the work of Dr. Harbitz. Among 172 autopsy cases examined by a morphometric technique, there was a marked decrease in the total Leydig cell mass with increasing age in individuals with

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benign hyperplasia, carcinoma of the prostate or atypical glandular proliferation. In patients with a histologically normal prostate, no such decrease with age was observed.

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STUDIES ON THE PATHOGENESIS OF BENIGN PROSTATIC HYPERTROPHY IN THE DOG

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Introduction

Three underlying assumptions about the pathogenesis of prostatic hypertrophy have been the basis for studies in our laboratory over the past seven years. The first is that the development of the hypertrophic prostate is androgen-dependent (1,2). It seems relatively clear that the disorder does not develop in the castrate, but it should also be recognized that it is not established whether testicular hormones play a causal role in this regard or act simply in permissive fashion. The second assumption is that the condition in the dog and that in man have a common pathogenesis. The fact that prostatic enlargement to the point of producing obstructive symptoms occurs commonly in dog and man is well documented. While there are clear-cut anatomical and histological differences between the conditions in the two species, there are also striking similarities in regard to the natural history and symptomatology, leading us to assume that both are conditions of unregulated growth. The final assumption is the logical extension of the first two. If the process is species specific (many animals do not develop the disease) and if it is endocrine-mediated, then some unique feature of steroid hormone physiology should be found that would explain the pathogenesis.

Comparative Studies of Dihydrotestosterone Formation

The initial studies evolved from measurements of 5α-androstane formation in androgen target tissues. In 1968–1969 it was recognized in several laboratories that dihydrotestosterone, the 5α-reduced derivative of testosterone, was the major steroid bound to nuclear chromatin in the prostate and some other androgen target tissues (3,4). Since that time a considerable body of evidence has accrued to indicate that dihydrotestosterone formation may be involved in many androgen actions (5,6). The concept that dihydrotestosterone formation might be involved in prostatic hypertrophy originated in a comparative study by Gloyna and Wilson of dihydrotestosterone formation in the prostates of eleven different animal species (7,8). In that study the rate of conversion of testosterone to dihydrotestosterone by tissue slices from the prostates of adult animals varied from high levels in rat, man, baboon, lion, and dog to virtually undetectable levels in species such as the bull and rabbit. It is well established that there is a marked species variation in the development of the prostate. When the rate of dihydrotestosterone formation was plotted against the weight of the prostate in the mature animal, expressed as g/kg of body weight, it was apparent that there is a reasonable linear correlation between dihydrotestosterone formation and the ultimate weight of the gland (Fig. 1). Furthermore, it was possible to show that in all species dihydrotestosterone formation is rapid during the early developmental stages of growth. For example, in the prostates of the puppy, kitten, and newborn calf there is little difference in the rates of

This work was aided by grants AM03892 and HD00107 from the U.S. Public Health Service.

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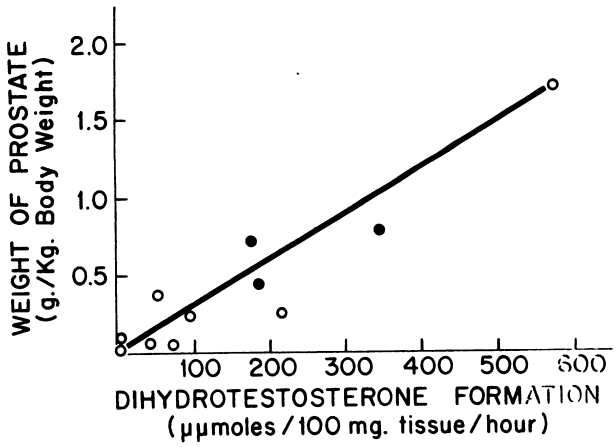

FIGURE 1. The relation between the rate of dihydrotestosterone formation by prostate slices and the weight of the gland in the adult animal. Reprinted from Wilson and Gloyna (8).

dihydrotestosterone formation. In dog prostate dihydrotestosterone formation remains high throughout life; there is little change in the activity in the cat, whereas the capacity virtually disappears in the prostate of the bull after growth has ceased (8). As shown in Fig. 2, a more detailed study of dihydrotestosterone formation as a function of age was made in rat and rabbit. The rate of formation remains fixed in the rat from birth to at least 36 weeks of age, whereas in the rabbit the capacity is lost just prior to 12 weeks of age as growth diminishes. Two alternate interpretations of the results were possible. On the one hand, it was conceivable that dihydrotestosterone formation is normally rate-limiting for prostatic growth and that unregulated production of dihydrotestosterone over many years might result in hypertrophy of the gland in susceptible species. On the other hand, it was equally possible that increased dihydrotestosterone formation was only a secondary accompaniment of prostatic growth that was induced by other factors. The unraveling of these alternate interpretations has required a great deal of time.

Dihydrotestosterone in the Hypertrophic Prostate

To explore the possibility that dihydrotestosterone formation might be causally linked to benign prostatic hypertrophy, a double isotope derivative technique was adapted for the measurement of tissue androgen content in normal and hypertrophic human prostates (9). A portion of these data are summarized in Fig. 3. There was no significant difference between the two types of gland in the content of testosterone at any age. However, on an average dihydrotestosterone content was greater

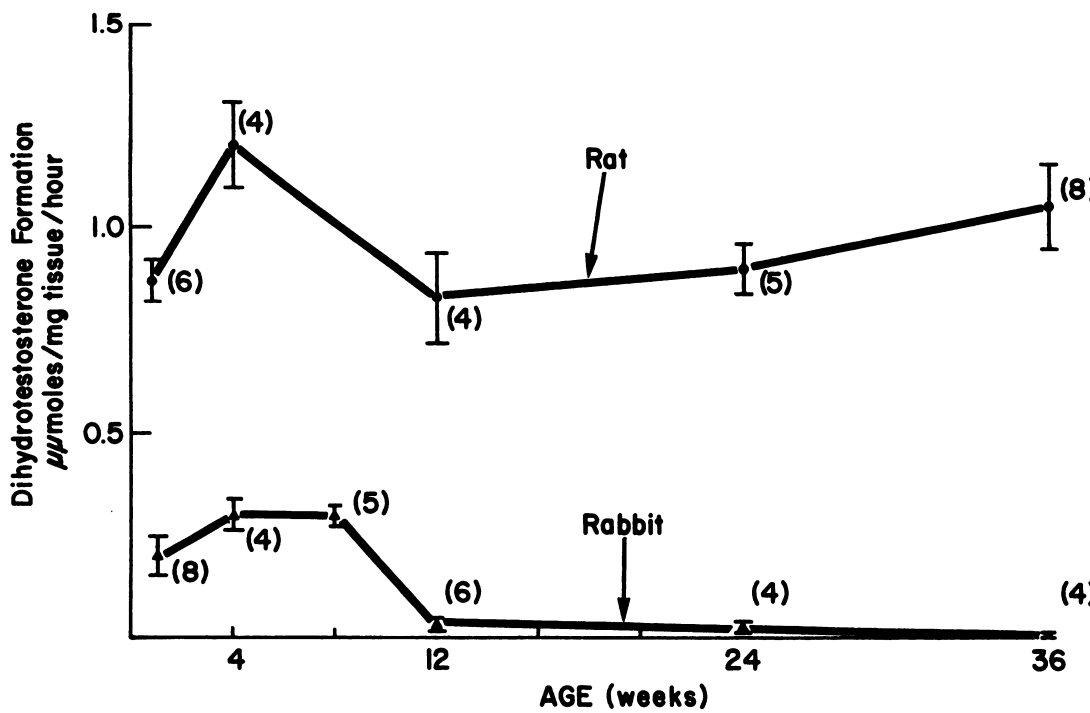

FIGURE 2. Dihydrotestosterone formation by slices of rabbit and rat prostate in relation to the age of the animals.

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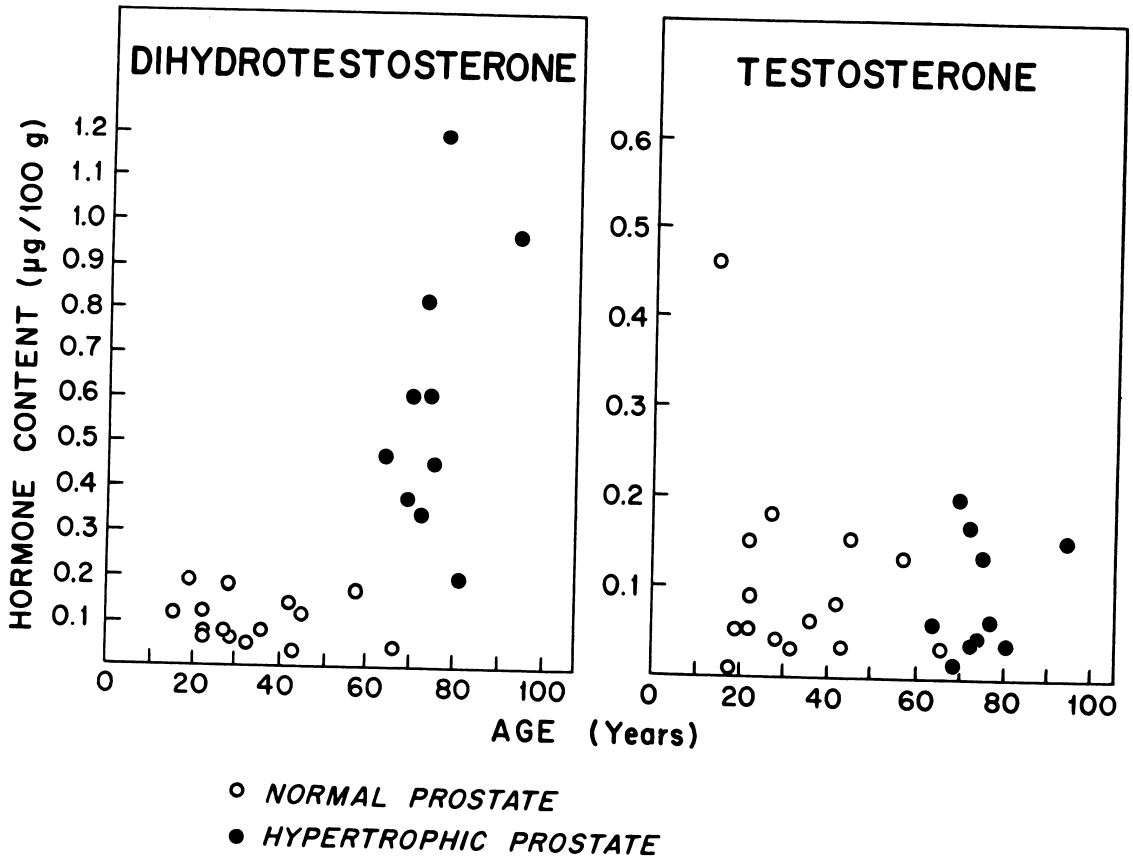

FIGURE 3. The relation between testosterone and dihydrotestosterone content of the prostate and age. Reprinted from Siiteri and Wilson (9).

in the hypertrophic tissue (0.60μg/100 g) than in the normal glands (0.13μg/100 g), and when these data were plotted in relation to the age of the subject it is clear that dihydrotestosterone content rises strikingly with age.

Prostatic hypertrophy in man begins in the periurethral area of the gland. When regional androgen concentrations within the gland were assessed, the content of dihydrotestosterone was found to be higher in the periurethral region of the normal gland than in the outer regions, and in three prostates with early concentric hypertrophy involving the periurethral regions only, dihydrotestosterone content in the involved portion of the gland was higher still; in the uninvolved portion dihydrotestosterone content was unchanged (9). However, dihydrotestosterone formation from radioactive testosterone was similar in normal and hypertrophied glands. Studies in immature, mature, and hypertrophic dog prostates gave findings much like those in man. There was little change in testosterone content with age, whereas the content of dihydrotestosterone was strikingly increased in the hypertrophic gland. In addition, no significant change in dihydrotestosterone formation from testosterone was noted between the three types of prostates (10). On the basis of these studies it was not possible to account for the dihydrotestosterone accumulation or to decide whether the hormone caused the hypertrophy.

Effect of Dihydrotestosterone on the Dog Prostate

The critical issue is whether dihydrotestosterone can cause prostatic hypertrophy in the castrate animal. To examine this possibility in detail, several studies have been undertaken. Young male dogs averaging 1½-2 years of age and with an average prostate of 2.8 g were divided into two groups. Control animals received triolein injections while the experimental group was castrated and given triolein containing no hormone or triolein with dihydrotestosterone or testosterone (75 mg/week). In the first study, the animals were killed after nine months; in the castrate animals treated only with

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triolein prostate weight had regressed to 1.8 g. In the testosterone-treated group average weight was 3.6 g, not significantly different from the control group. However, in the dihydrotestosterone group, the weight was nearly doubled, averaging 6.6 g (10). Histologic examination of the glands was of interest (Fig. 4). In the control tissue the acini were lined with columnar epithelium and surrounded by thin fibrous septae. After castration virtually all glandular elements disappeared, leaving only undifferentiated stroma. The tissue from testosterone-treated animals appeared normal, whereas the prostate from the dihydrotestosterone-treated animal showed large, irregular alveoli, a finding characteristic of canine prostatic hypertrophy (11). The results were considered compatible with a possible etiological role for dihydrotestosterone in the production of prostatic hypertrophy in the dog.

However, we had not succeeded in producing true prostatic hypertrophy in the castrated dog. The minimal criterion for the diagnosis in the dog according to Berg (11) is a gland weight of 15 g which was not obtained in the 9 month study. Nor did the dogs develop obstructive symptoms. For

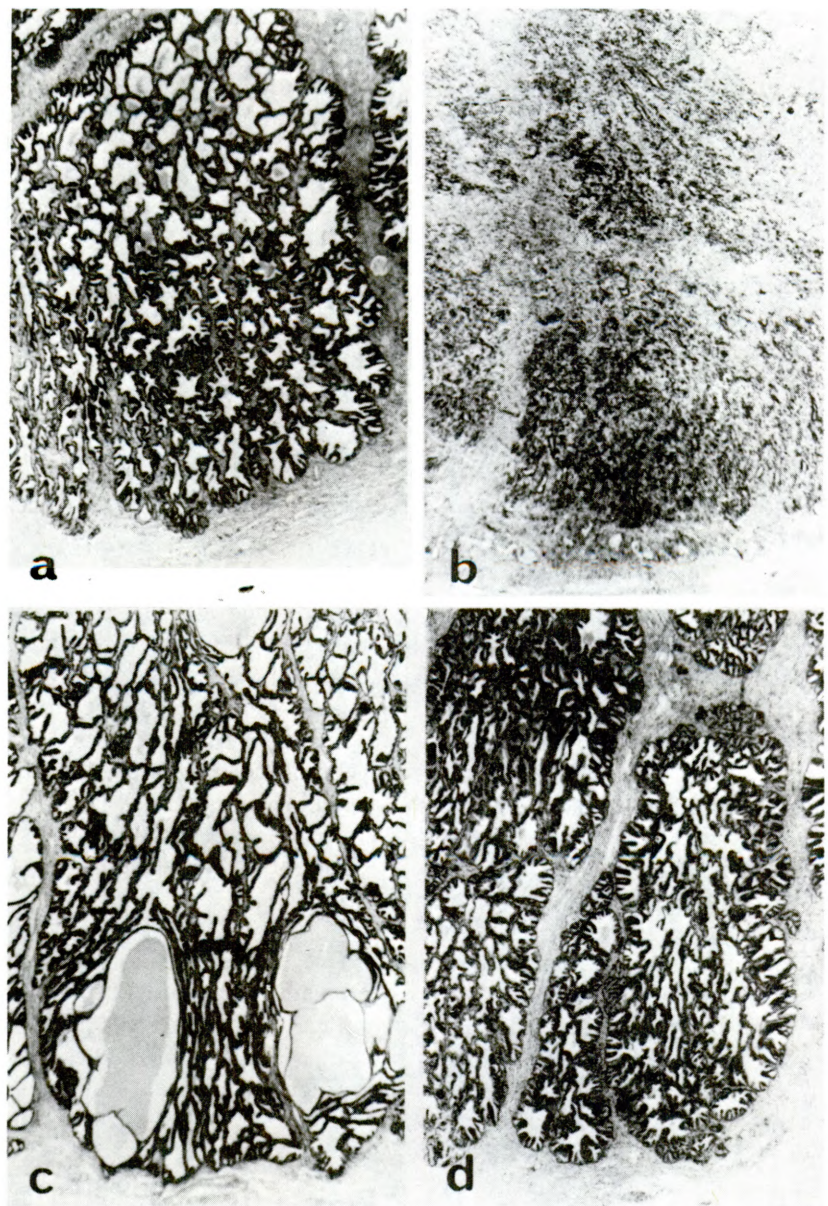

FIGURE 4. The effect of dihydrotestosterone and testosterone on the histology of the dog prostate. a.) Control prostate, b.) prostate from a castrated dog, c.) prostate from a castrated dog treated with dihydrotestosterone for nine months, and d.) prostate from a castrated dog treated with testosterone. Hematoxylin and eosin stain X 40. Reprinted from Gloyna, Siiteri, and Wilson (10).

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these reasons, two long-term studies were undertaken in which young control and castrated dogs were given either triolein or pharmacological doses of testosterone or dihydrotestosterone (75 mg/week) for about two years (Table I and Ref. 12). During this study two of the control dogs developed early prostatic hypertrophy (15.5 and 19.4 g), and the average weight of the prostate in the control group was 14 g. In the castrate controls prostatic weight was identical to that in the 9 month study (1.8 g), while the weights of the glands in the castrate animals given dihydrotestosterone or testosterone did not increase significantly above that seen with 9 months of treatment. The failure to produce hypertrophic growth was considered to be significant since both testosterone and dihydrotestosterone treatments produced dihydrotestosterone concentrations in the prostate comparable to the control group where hypertrophy developed in half the animals (1.0 and 0.6 μg vs. 0.6 μg per hundred grams weight). Testosterone values in testosterone-treated and control dogs were also similar (0.2 vs. 0.1 μg/100 g). Thus, despite the fact that this hormonal regimen caused an almost exact duplication of the content of these two hormones in the treated castrate as in the hypertrophic glands, growth in the treated castrate was not as great as in the controls, and no true hypertrophy developed. Furthermore, the failure to induce prostatic hypertrophy in the castrate by dihydrotestosterone treatment could not be explained by an inadequate duration of the study since during the same time period half the control dogs developed hypertrophy.

One potential explanation for the failure of exogenous androgens to induce prostatic hypertrophy in the dog was that the mode of delivery of the hormone to the gland was critical, e.g. the possibility was considered that androgen delivered directly to the gland via the seminal fluid might play a critical role in the pathogenesis of prostatic hypertrophy. To be certain, therefore, that all testicular hormones reached the gland via the circulation, vas duct ligation was performed on six dogs with prostatic hypertrophy (Table II). After six months there was no difference in the average weight of the glands between treated and control groups (28 and 24 g), and no significant difference was found between the two groups in the content of testosterone and dihydrotestosterone in plasma and prostate (12). It is clear that vas duct ligation has no effect on the hypertrophic prostate, and the conclusion has been drawn that any hormone that reaches the gland via the seminal fluid does not play a significant role in regulating gland growth. As the result of these various studies, it was concluded that some hormone or hormones from the testis other than testosterone or dihydrotestosterone must be critical for the development of prostatic hypertrophy in the dog. Preliminary results indicate that 3α-

TABLE I—Effect of testosterone and dihydrotestosterone treatment for two years on the dog prostate (Reprinted from Wilson, Gloyna and Siiteri, Ref. 12)

Group	Number	Treatment	Prostate Weight g	Hormone Content μg/100 g or 100 ml			
				Testosterone		Dihydrotestosterone	
				Prostate	Plasma	Prostate	Plasma
I Control	4	Triolein	14	0.09	0.10	0.55	0.01
II Castrate	4	Triolein	1.8	0.07	0.01	0.10	0.01
III Castrate	4	Dihydrotestosterone	6.6	0.02	0.01	0.58	0.12
IV Castrate	4	Testosterone	3.6	0.15	0.68	0.99	0.08

TABLE II—Effect of vas duct ligation on the hypertrophic dog prostate (Reprinted from Wilson, Gloyna and Siiteri, Ref. 12)

Group	Number	Prostate Weight g	Hormone Content μg/100 g or 100 ml			
			Testosterone		Dihydrotestosterone	
			Prostate	Plasma	Prostate	Plasma
Control	11	24.4	0.14	0.20	0.40	0.02
Vas duct ligation	6	28.4	0.16	0.23	0.40	0.00

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androstanediol is more potent than dihydrotestosterone in inducing prostatic growth in the castrated dog.

What Regulates Dihydrotestosterone Formation in the Prostate?

If dihydrotestosterone formation is not rate-limiting in prostatic growth, then the question may be posed as to why the activity of the 5α-reductase enzyme in prostate parallels the growth rate in the gland. To clarify this issue, a variety of steroids were administered to castrate rats for 4 days, and the effects on prostate weight and 5α-reductase activity were assessed (Fig. 5 and Ref. 13). The group of hormones were chosen to include steroids that are potent substrates for the 5α-reductase but are weak androgens (20α-hydroxy-preg-4-en-3-one, progesterone, and epitestosterone), steroids that are potent androgens but are neither substrates nor inhibitors of the 5α-reductase (dihydrotestosterone and 3α-androstanediol), steroids that are neither androgens nor substrates for the 5α-reductase (5β-dihydrotestosterone and 3β-androstanediol), and steroids that might be expected to play a physiological role in the androgen-mediated growth of the prostate (testosterone, androstenedione, dihydrotestosterone, and 3α-androstanediol). As expected, these various steroids produced widely divergent effects on mean ventral prostate weight and on the activity of the enzyme; the relationship between 5α-reductase activity and the change in prostate weight resulting from the various treatments is described by a regression line with a correlation coefficient of 0.93 (13). There is no evidence for substrate induction of this enzyme since such potent substrates as epitestosterone, progesterone, and 20α-hydroxy-preg-4-en-3-one were ineffective in enhancing enzyme activity. It is apparent from

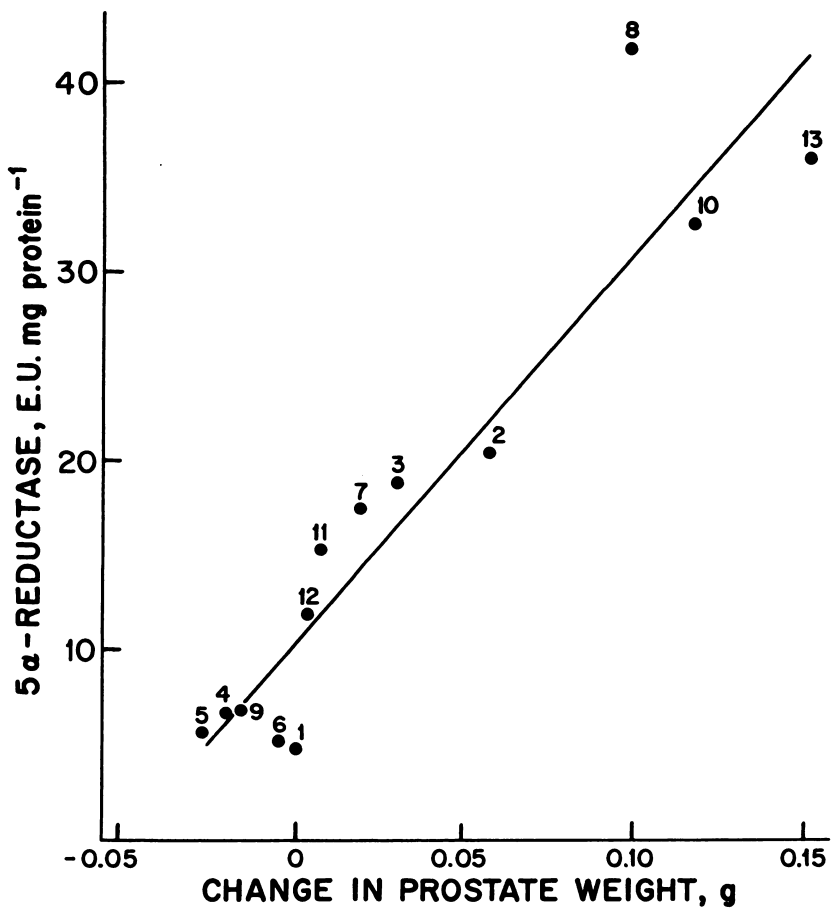

FIGURE 5. Relation between androgen-induced growth of the ventral prostate and 5α-reductase activity. 1. Castrate control, 2. testosterone, 3. androstenedione, 4. epitestosterone, 5. 20α-hydroxypreg-4-en-3-one, 6. progesterone, 7. androsterone, 8. 3α-androstanediol, 9. 3β-androstanediol, 10. dihydrotestosterone, 11. androstanedione, 12. 5α-dihydrotestosterone, 13. testosterone propionate. Reprinted from Moore and Wilson (13).

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these data that the ability to cause growth is correlated closely with the capacity to enhance 5α-reductase activity.

Further evidence that activity of the 5α-reductase enzyme is a manifestation or marker of androgen-mediated growth has been obtained from unpublished studies of prostate growth in the rabbit. If dihydrotestosterone formation were rate-limiting in growth, then administration of the hormone to the rabbit at an age when dihydrotestosterone formation in prostate has ceased (Fig. 2) should induce more growth than an equal amount of testosterone. However, even in pharmacological dosages both hormones are equally ineffective in increasing gland size in the adult rabbit (R.E. Gloyna and J.D. Wilson, unpublished results). Thus, the conclusion seems warranted that 5α-reductase activity in the prostate of several species is a manifestation rather than a cause of prostatic growth.

Conclusion

On the basis of the evidence at hand the conclusion seems inescapable that some testicular hormone or hormones other than testosterone or dihydrotestosterone is the cause of prostatic hypertrophy in the dog. This does not imply that such a factor need work directly on the gland, nor does it follow that such a factor must necessarily be a steroid hormone. However, several other steroid hormones are secreted by the testis, including estradiol (14) and 3α-androstanediol (15), and the effects of these hormones, alone and in combination with others, on prostatic growth in the dog are now being tested. Until the testicular product is defined it will not be possible to determine whether the species specificity in the occurrence of prostatic hypertrophy is due to some unique character of the testicular secretion or to some distinctive aspect of the recognition/receptor machinery itself in the prostates of these two species.

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DISCUSSION

D. S. COFFEY: Bruchovsky has shown if you give radioactive androstanediol or DHT to a rat an identical amount of androstanediol was recovered in the cytosol. Furthermore, some 90% of the label in the nucleus is DHT under both conditions. If that

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were also true in the dog, up-take into the nucleus alone could not explain the induction of BPH in the dog with androstanediol. I wonder if Dr. Ofner or maybe Dr. Eik-Nes, who have studied the metabolism of steroids in the dog prostate, have information on how androstanediol is handled vs. DHT?

P. OFNER: I have some additonal information on the disposition of the pertinent four C_{19}-steroids in explants of canine prostate and human prostate adenoma. Most of the morphologic features of canine prostate can be maintained in serum-free *medium* for three days, and when ^{3}H-testosterone is the substrate, 5α-reduced 7-hydroxylation products are the major terminal radiometabolites in 21 hour cultures (Ofner and Vena, Abstract 498, Program of the 57th Annual Meeting of the Endocrine Society, 1975). The 7-hydroxylated products also accumulate in the explants and medium when the three 17β-hydroxysteroid metabolites of ^{3}H-testosterone are the substrates. Adequate morphologic maintenance of human prostate adenoma nodules in 21 hour cultures has not been achieved, but in contrast to ^{3}H-testosterone disposition in canine prostate cultures, 5α-dihydrotestosterone is the principal radiometabolite, and the level of hydroxylation products is substantially lower. Thus the 3β-hydroxysteroid oxido-reductase and hydroxylase activities of the prostate may have an important role in regulating the biological effects of intracelluar androgens. The greater effectiveness of 3α-diol in the dog may be explained by slower prostatic conversion of the 3α-diol to 3β-hydroxysteroids or to its less active hepatic metabolism and excretion than 5α-dihydrotestosterone. Somehow the 3α-diol must find its way to the gland and exert its effects *per se* or as generated 5α-dihydrotestosterone.

S. G. KORENMAN: How are DHT and androstanediol transported in blood?

OFNER: According to Corvol and Bardin (Biol. Reprod. *8*: 277, 1973) sex hormone binding globulin is absent from canine blood. However, Carstensen et al (J. Steroid Biochem. *5*: 757, 1974), using a two-phase equilibrium partition procedure, found a specific testosterone-binding globulin in the male dog which does not bind estradiol-17β and is not identical with transcortin. I suppose canine transcortin will also have a certain testosterone-binding capacity.

H. BRENDLER: Does the administration of DHT or androstandiol result in an acceleration of BPH development in the intact dog?

J. D. WILSON: It would be very interesting if one could give exogonous hormones in the presence of an intact testis and accelerate the production of BPH.

O. HECHTER: If you give the 3α-diol to a rat, do you get BPH?

WILSON: In short term experiments 3α-diol is slightly more androgenic than dihydrotestosterone. We have not studied the long term effects.

N. BRUCHOVSKY: We've been studying the effects of dihydrotestosterone on proliferative growth in different organs, and we became interested in the observation that dihydrotestosterone is elevated in benign prostatic hyperplasia. We examined both 5α-reductase activity and 3-hydroxy steroid dehydrogenase activity in 18 specimens of hyperplastic prostate obtained by abdominal surgery and in 7 normal prostates obtained within 5–6 hours following death. In the nodules from hyperplastic prostate the 5α-reductase activity was at least 3 times that in the normal prostate. On the other hand, the deydrogenase activity was equal in the nodules and the normal tissues. This asymmetry between the reductase and the dehydrogenase activities could account for the net increase in dihydrotestosterone concentration. We have also looked at the conversion of androstanediol to dihydrotestosterone in prostate and in skin. Unlike the rat prostate, human tissues do not convert 3α-androstanediol to dihydrotestosterone very actively.

WILSON: I agree with the need to re-examine quantitatively the various enzymes involved in androgen metabolism in the human prostate. However, one should be cautious about using autopsy material. In our hands 5α-reductase is very unstable. What is needed is a quantitative micro enzyme assay so that these various enzymes can be measured in the amount of tissue obtained from needle biopsy.

J. T. GRAYHACK: The main thrust of this meeting has been the concept that the understanding of testosterone and its various metabolites will eventually provide a solution to the problem of BPH. Certainly knowledge regarding testosterone metabolism is important, but there is no explanation for the paradoxical accumulation of androgens in the periurethral gland in the presence of a decrease in systemic level. There is some evidence that there are substances present in the aging male that are not androgenic but that stimulate the prostate. We studied about 200 specimens of seminal vesicle at autopsy. The seminal vesicle in man is rarely the site of pathologic change and it is an accessory sex gland that might reflect the hormonal

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milleau in the aging male. Interestingly, the fructose content of the seminal vesicle fluid decreases with age much as the androgen studies have shown today, but the weight of the vesicle was maintained at all ages, suggesting that there might be some non-androgenic accessory sex gland stimulating substance. I think that we need to keep this possibility in mind in our future efforts.

L. M. FRANKS: There might be age associated intracellular changes which make the cells particularly sensitive so that they respond in an abnormal way to pre-existing stimulus. If, for example, androstanediol really acted as a trigger on a cell that was altered by some age associated change, you might continue to get stimulation by some hormones that are already present. Do you have to continue to treat with androstanediol, or if you treat a short time in a dog that is already conditioned by time, will the thing then go on?

WILSON: I don't know. Our dogs are all approximately two years of age when the experiment begins.

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EXPERIMENTAL APPROACHES TO BENIGN PROSTATIC HYPERTROPHY: ANIMAL MODELS UTILIZING THE DOG, RAT, AND MOUSE

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Research into the etiology of benign prostatic hypertrophy has been hampered by the difficulty in reproducing this disease in experimental animals. In the search for a suitable experimental model, wide use has been made of several animal species. In the rat and the mouse hormonal manipulation can induce glandular and stromal changes that are histologically similar to those found in benign prostatic hypertrophy, and in the aged dog a form of prostatic hyperplasia occurs spontaneously. In this review the comparative aspects of these models will be presented.

Rat and Mouse

In the rat the prostate gland is composed of discrete paired ventral lobes, a dorsolateral group of prostatic acini and ducts that surround the urethra and base of bladder and paired coagulating glands. The prostate of the mouse is similar to that of the rat, except that the ventral and lateral lobes are relatively less well developed. Embryologically, Price has demonstrated that the dorsal lobe of the rat prostate is homologous with the dorsal (posterior) lobe in man and that the lateral lobes correspond to lateral lobes in man (1). Although the coagulating glands may be homologous with man's middle lobe, Price does not consider the ventral lobe of the rat prostate to correspond with the ventral lobe in man (1).

Histologically, the adult prostate in the rat is composed of acini of different sizes. Each acinus is lined by columnar epithelium, which rests on a basement membrane, and is surrounded by a sheath of connective tissue and smooth muscle cells provided with nerve fibers. The ventral prostate contains several phosphatases and has a remarkably high concentration of citric acid (2). However, it completely lacks fructose (3). The dorsolateral prostate differs from the ventral lobes because it produces fructose in addition to citric acid (3). With aging, the prostates of both the rat and the mouse undergo atrophy with a decrease in the height of the epithelium, loss of papillae, and prominence of the periacinar collar of stroma (4). Although spontaneous adenocarcinoma of the prostate has been described in aged rats (5,6), benign prostatic hyperplasia does not occur. However, prostatic hyperplasia has been reported in one strain of female mice.

A prostate is present in some female rats, and the incidence of prostates in females can be increased by selective breeding. In the multimammate mouse, *Mastomys erythroleucus* Temm., a well developed prostate is present in all females (7). This prostate consists of paired lobes which resemble the ventral prostate in the male both anatomically and histologically. Snell and Stewart have reported the development of proliferative hyperplasia of the prostate in four female *Mastomys* that were sixteen to thirty-three months of age (8). These animals also had papillary cystic hyperplasia of the endometrium, hyperplasia of the mammary gland, brown degeneration of the adrenal, carcinoma of the glandular stomach, and granulosa cell proliferation of the ovary. Because alterations in the endocrine and sex organs are not unusual in female *Mastomys* that have a normal prostate gland, the authors were not able to assess the significance of the changes in other endocrine organs associated

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with prostatic hyperplasia. Unfortunately, further studies have not been reported.

Because spontaneous prostatic hyperplasia occurs rarely in the rodent, there have been many experimental attempts to produce it. In 1933 Lacassagne produced enlargement of the posterior and lateral lobes of the mouse prostate with folliculin (oestrin) injections (9). In this study obstruction of the bladder neck occurred with resultant urinary retention and hydronephrosis. Burrows and Kennaway noted similar findings by applying estrin to the skin of male mice (10). In this study there was cystlike distention of the prostatic alveoli which were lined with keratinized squamous epithelium. Since these pioneering observations, it has been well demonstrated that distinct site and species differences exist in the response to estrogen. Bern has reported that the metaplastic response of the male accessories to estrogen may be arranged according to extent as follows: mouse>guinea pig->rabbit>rat (11). Although the ventral prostate does not show metaplasia, extensive squamous metaplasia and keratinization do occur in the coagulating gland of the mouse.

More recently, Fingerhut and Veenema have utilized estrogen-induced prostatic obstruction as a model for the study of benign prostatic hyperplasia in mice (12). They implanted pellets containing 1.25 mg of diethylstilbesterol into three month old male mice of the Strong A/J strain. After three months, although the overall weight of the posterior urethral and bladder neck tissue did not show an increase over the control value, the treated mice developed urinary retention, hydroureter, and hydronephrosis. Histologically, there were areas of stromal hyperplasia consisting of whorl-like nodules of connective tissue and smooth muscle (Fig. 1). In addition there were numerous cystic, dilated, prostatic acini that were lined by flattened squamous epithelium and were filled with degenerated keratinized cells that had desquamated into the lumen. In a subsequent study, these same authors demonstrated that in treated mice the adrenals and pituitary glands were increased in size and weight (13). Following bilateral adrenalectomy, the prostate, posterior urethra, and bladder neck of these treated animals approximated a more normal appearance and urinary retention was relieved. Although it is well known that estrogen administration induces adrenal hyperplasia in rats by increasing the release of corticotropin-releasing factor and stimulating the synthesis of ACTH (14), the mechanism by which adrenalectomy reduced urinary retention is unclear.

Caine and Superstine repeated these studies using male albino mice of the Hebrew University strain (15). They found urinary retention in only 17% of the treated animals and were unable to reverse this effect using a progestational agent, 17-α-hydroxyprogesterone caproate, which effectively reduces the weight of the ventral prostate in rats. Thus, although it is clear that stilbesterol treatment of mice will induce bladder neck obstruction and hydroureteronephrosis, it is unclear whether this disease bears any relationship to prostatic hypertrophy in man. The obstruction induced in mice occurs without an increase in weight of the prostate and is characterized by both fibrostromal hyperplasia and marked squamous metaplasia with dilated obstructed acini containing large amounts of keratin debris.

There have been other attempts to produce prostatic hyperplasia in the rodent using androgens. Following the discovery that dihydrotestosterone is the major androgenic metabolite concentrated in the nuclei of the prostate (16), several lines of evidence suggested that a continued high rate of conversion of testosterone to dihydrotestosterone, within the prostate gland, may be involved in the pathogenesis of prostatic hypertrophy. Gloyna and Wilson found that in eleven animal species, the rate of conversion of testosterone to dihydrotestosterone by prostatic slices correlated with the ultimate size of the gland and that man and the dog had high rates of conversion (17,18). In addition, Siiteri and Wilson demonstrated that in human hypertropic prostatic tissue dihydrotestosterone concentrations were almost five times higher than in normal prostatic tissue (19). Based on these findings, Gloyna and Wilson attempted to induce prostatic hyperplasia in the rat with the long term administration of dihydrotestosterone (20). Treatment with dihydrotestosterone for as long as seven months, caused prostatic growth almost twice as great as equivalent amounts of testosterone, and when these prostates were examined histologically there was an increase in fibrous connective tissue and a decrease in the average height of the acinar cells in two of five animals (Fig. 2) (18). In another study, McGuire et al, treated rats for six to fifteen weeks with dihydrotestosterone (21). In this study, the weight of the ventral prostates was no greater than in testosterone treated animals, and when a Zeiss intergrating reticule was used for the purpose of quantitating the proportion of epithelium and stroma in histologic sections, there was no indication of stromal hyperplasia. Consequently, it appears that in the rodent neither estrogen nor an-

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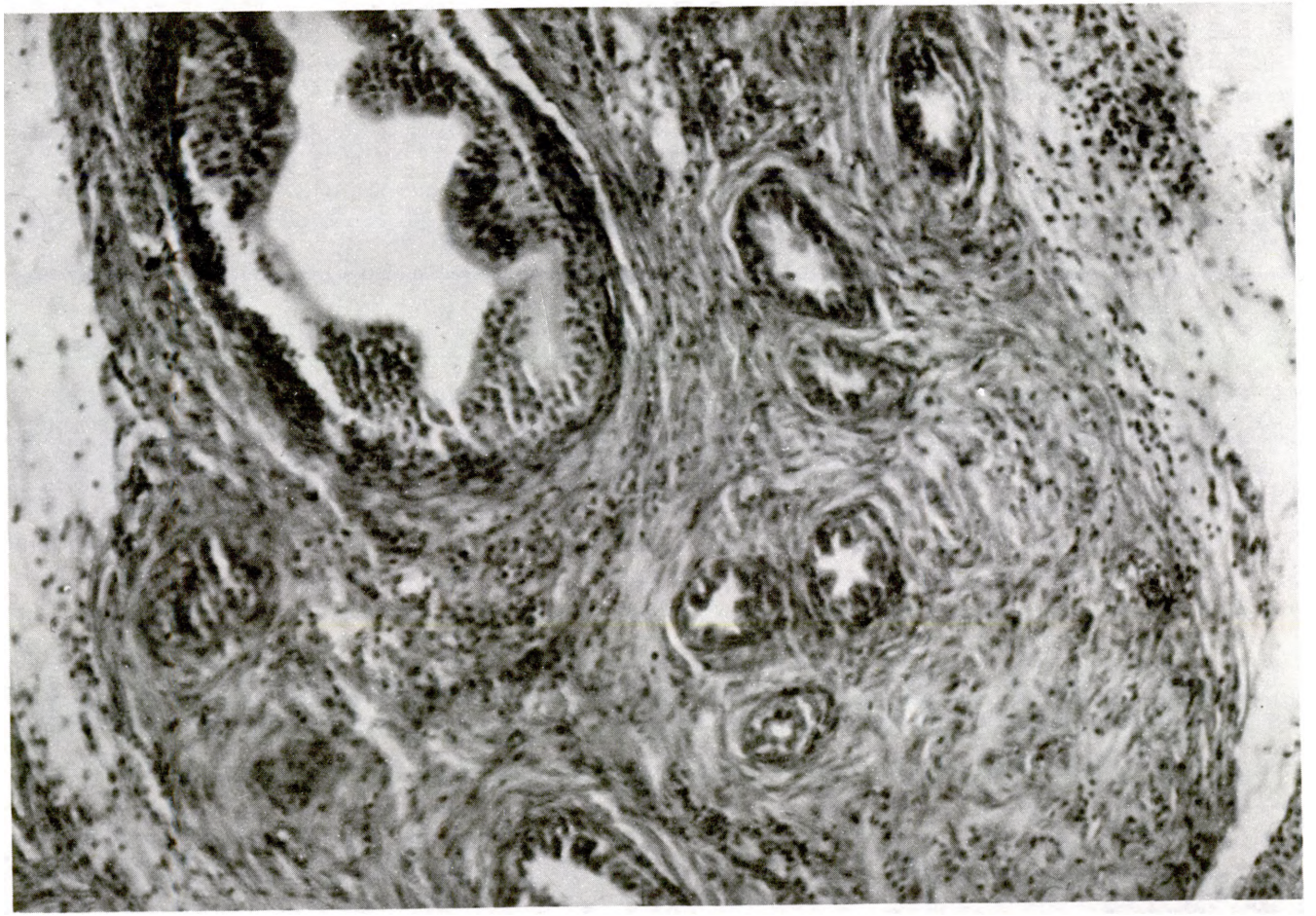

FIGURE 1. Prostate of a 6 month old Strong A/J mouse following treatment with diethylstilbestrol (x250). (Courtesy of Dr. Bruno Fingerhut).

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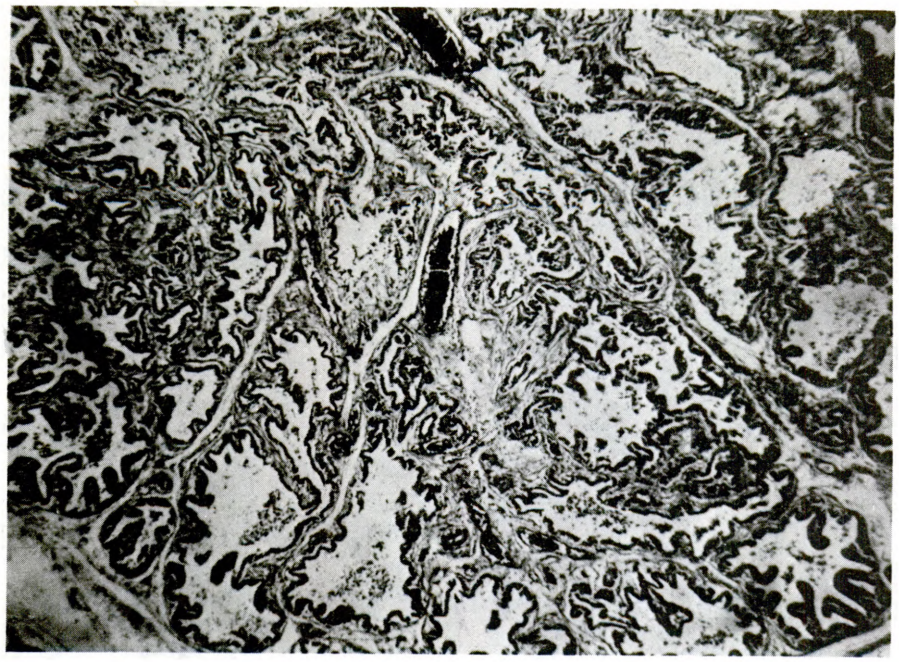

FIGURE 2. Ventral prostate of rat treated with dihydrotestosterone for seven months. (Courtesy of J. D. Wilson, M.D., Dallas, Texas).

drogen will reproduce the fibrotic prostatic enlargement that is characteristic of the disease in man.

The Dog

The dog has neither seminal vesicles nor bulbourethral glands, and consequently the prostate is its only well developed accessory sexual gland. In the dog the prostate is relatively large, completely surrounds the urethra, and is normally situated in the middle of the pelvic inlet resting on the cranial edge of the symphysis pubis. The prostate is almost globular in form, yellow in color, and has a distinct dorsal median furrow. Histologically, it is composed of branching secretory acini and ducts that radiate on all sides of the prostatic urethra. In the adult dog, Berg has reported that the size and weight of the normal prostate are: length: 1.9–2.8 cm; width: 1.9–2.7 cm; height 1.4–2.5 cm; and weight 4.0–14.5 gm. or 0.21–0.57 gm/kg (22). One exception, however, is the Scottish terrier, in which the prostate weight normally is four times as great as the mean weight of other dogs (23).

Man and the dog are the only common mammals that spontaneously develop benign prostatic hypertrophy. The natural history of this condition in the dog is remarkably similar to that in the human. The disease is common in dogs over the age of five and in men beginning in the fifth decade (24). In both, early castration prevents it occurrence, and normal testicular function is necessary for its development (25,26). The size of the enlarged prostate in both man and the dog are remarkably similar (40–100 grams), and in both the concentration of dihydrotestosterone in hyperplastic tissue is five times higher than in the normal prostate (19,27). Although the disease in the dog is remarkably similar to benign prostatic hypertrophy in man, canine prostatic hyperplasia does have two distinctive features: the histological picture is predominently hyperplasia of the glandular elements with only sparse fibromuscular stroma, and the major symptom in the dog is constipation rather than urinary tract obstruction (24). Less commonly, dogs may develop interference with micturition. In the dog, regression of the hyperplasia occurs rapidly following orchiectomy or treatment with antiandrogens (28–31). In man, although conflicting data have been reported, similar but less impressive results have been achieved with these forms of treatment (32–34).

In canine prostatic hyperplasia, the entire gland is enlarged, often immensely (35), due to an increase in both the size and number of glandular acini (36). The epithelial cells lining the acini in-

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crease in height but they increase much more in numbers, forming papillary projections into the lumen. Accompanying this glandular proliferation there is an augmentation of the fibrous tissue and smooth muscle of the septa and supporting tissues. Occasionally, this increase of the supporting tissue is the predominent change, but in the great majority of cases it merely keeps pace with the epithelial hyperplasia (36). Although the epithelial hyperplasia is often diffuse (Fig. 3b), frequently acini dilate forming cysts (Fig. 3c). These cystic acini are irregular in size and are lined by an epithelium which has become pressed and flattened. The interlobular connective tissue is variable in amount and may exist as broad sheets with some extension into the intralobular stroma. Berg has stated that although some of the histological changes of prostatic hyperplasia may be present in glands that are not enlarged, the term hypertrophy (or hyperplasia) should be limited to cases in which enlargement of the prostate gland is clinically recognizable (26). Based on his findings in normal dogs, he states that in adult dogs, no normal prostate weighs more than 14.5 grams, and if the length, width, or height of the prostate exceeds 3–3½ cm the gland is most likely pathologically involved (22). Occasionally, prostatic enlargement may occur secondary to stimulation by estrogens. This enlargement is characterized by extensive squamous metaplasia of the prostatic ducts, dilated obstructed acini and fibromuscular hypertrophy. These changes can be induced by estrogen treatment (37) or can occur naturally in dogs with estrogen secreting Sertoli cell tumors (38). It is important to recognize that prostatic enlargement due to epithelial hyperplasia never occurs in dogs with Sertoli cell tumors (38,39), and that treatment with estrogen produces regression of canine prostatic hyperplasia (26).

Summary

This paper have reviewed various experimental models for the investigation of benign enlargement of the prostate. In the rat and mouse, spontaneous benign enlargement does not occur, and, therefore, various hormonal manipulations have been utilized in an attempt to reproduce this disease in rodents. In most respects, these attempts have failed to produce prostatic enlargement with the characteristics that typify benign prostatic hypertrophy in man.

In the dog, prostatic hypertrophy occurs spontaneously with aging and in many respects is similar to the disease that occurs in man. Although there are anatomical and histological differences between the processes in the two species, this appears to be the best experimental model currently available in animals.

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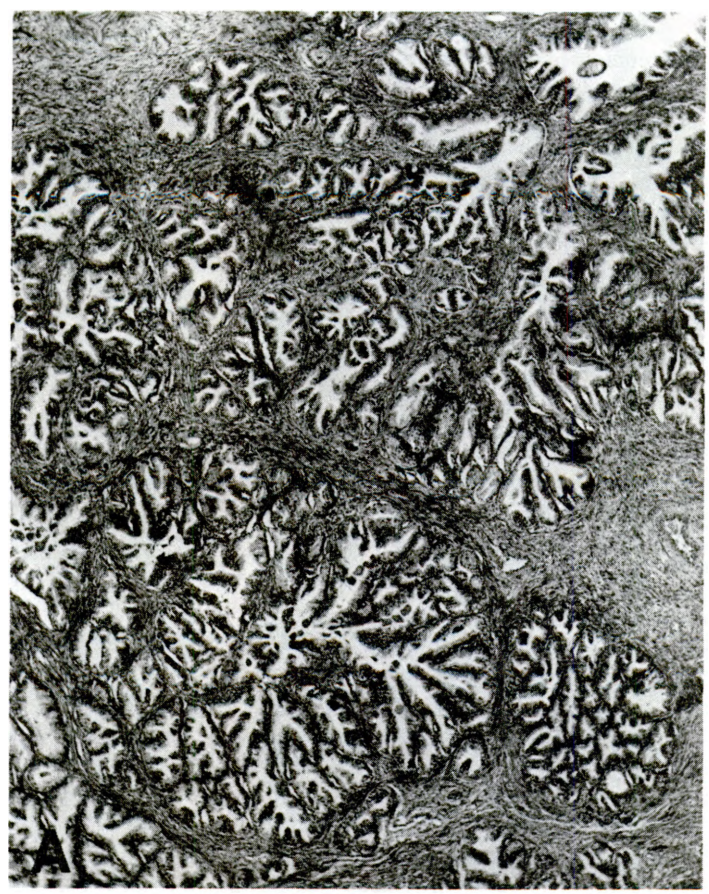

FIGURE 3. a. Prostate with benign hyperplasia—man (x35)

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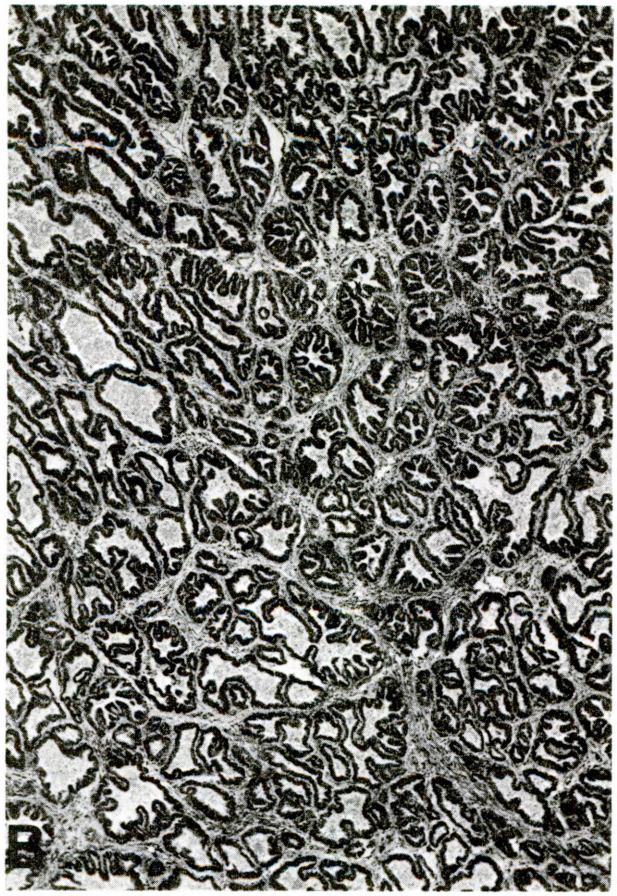

FIGURE 3. b. Prostate with diffuse glandular hyperplasia—dog (x35).

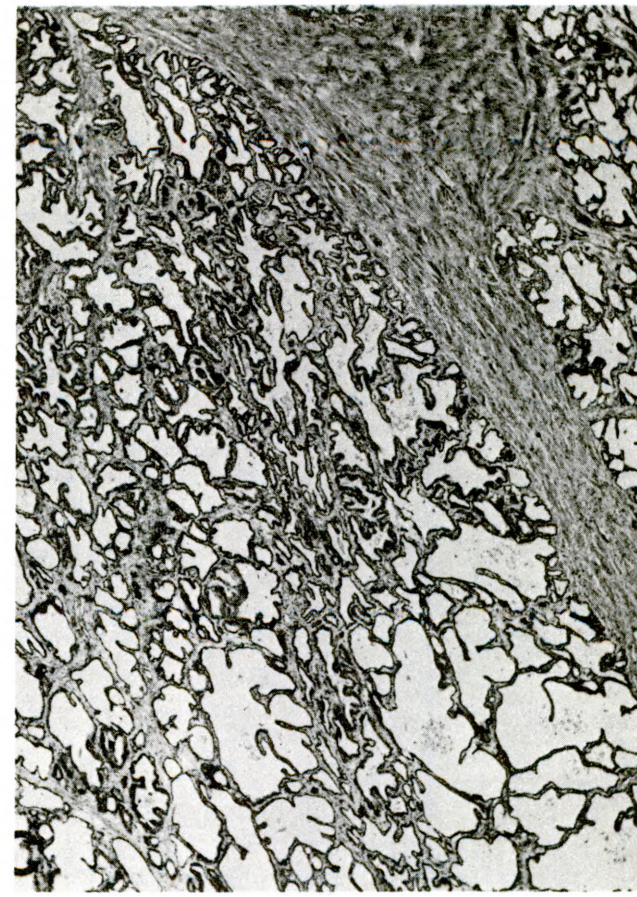

FIGURE 3. c. Prostate with cystic hyperplasia—dog (x35).

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EFFECTS OF AGE, CASTRATION AND STILBESTROL TREATMENT ON THE GUINEA PIG PROSTATE AND ASSOCIATED STRUCTURES

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There is at present no satisfactory animal that can be used for the study of benign prostatic hyperplasia as it occurs in man. Unsuccessful attempts to reproduce the human condition have been made in laboratory rodents—and these almost entirely using rats and mice (1,2). The guinea pig has been largely neglected although its prostatic gland is simpler in structure than that of the rat or mouse and although there are many aspects of its endocrine physiology and reproductive behaviour that differ from those of the other rodents and resemble more closely those in man.

We therefore studied the structure of the guinea pig prostate during ageing and endocrine treatment. In the outcome we found that stilbestrol treatment accelerated or reproduced changes seen in the aged prostate, particularly epithelial metaplasia. Fibroepithelial tumours developed in stilbestrol-treated animals but only in the prostatic utricle.

A further unexpected finding was that the atrophy of the gland following castration was not permanent: normal structure was restored after about a year in many cases.

MATERIALS AND METHODS

In 3 separate experiments, young (2 weeks old) and adult (about 10 months old) animals from our closed colony were castrated, treated with stilbestrol, or castrated and treated with stilbestrol. There were 184 experimental and 118 control animals.

Stilbestrol was administered by the subcutaneous implantation of a 15mg tablet containing 1% magnesium stearate (Implantin, Burroughs Wellcome but no longer available) which was renewed every 2–4 months.

The tablets were not weighed when the animals were killed or died, but on the average a tablet lasted about 9 months. Taking into account the higher rate of absorption immediately after implantation and the supplementary absorption from subsequent tablets later, it is probable that the daily absorption would be about 100 μg. Individual variation in absorption is not important with such grossly unphysiological overdosage. For histology paraffin sections were prepared from formalin-fixed material and stained with Erhlich's haematoxylin and eosin or a combined periodic Schiff, alcian blue stain.

RESULTS

Prostate weight

Since the study was primarily histological the prostate was not dissected free. The weights were recorded after fixation (Table I) and comprise both inner and outer prostate plus that portion of the urethra containing the verumontanum.

The most striking change was the gradual restoration of normal prostate weight after castration. Stilbestrol reduced prostate weight to castrate levels that were maintained while treatment continued both in intact and in castrate animals. When

*Dr. Chesterman died on April 3rd, 1973.

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TABLE I—Mean Weight of Prostate (g ± SEM)

Group	Expt. No. *	Age in years 0–1/2	1/2–1	1–2	2–3	3–4	4–5
N		2.5 ± 0.2	3.6 ± 0.3	4.5 ± 0.4	4.2 ± 0.4	4.3 ± 0.3	5.5 ± 0.7
C	1	0.9 ± 0.1		2.8 ± 1.0	3.1 ± 0.6		
C	2			2.2 ± 0.7	3.0 ± 0.3	3.7 ± 0.7	6.1 ± 1.5
S	1	1.0 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.2 ± 0.0		
	2				3.6 ± 0.4	1.8 ± 0.2	2.4 ± 0.2
CS	1	1.0 ± 0.1	2.1 ± 0.7	1.8 ± 0.6			
	2			2.2 ± 0.4	3.1 ± 0.2		2.2 ± 6.3

*Castration and stilbestrol treatment at 2 weeks of age (Expt. 1) or 10 months (Expt. 2). No. of animals in each group as given in Table 3
SEM = standard error of mean

treatment was stopped in 2 animals normal prostate weight (5.8g) was regained.

The normal prostate

The structure of the normal guinea pig prostate has been described by Rauther (3). It is simpler than that of some other rodents and consists of only two gland groups (Figs. 1 and 2). There is an inner group of glands, the coagulating gland closely applied to the seminal vesicles and a surrounding outer group which is divided into ventral and dorsal lobes, but this is not always apparent in transverse sections.

The ducts of both groups converge to pass through the urethral wall and open on to the verumontanum which also contains the ducts of the seminal vesicles and vasa deferentia and the bicornuate prostatic utricle. The structures of the verumontanum are surrounded by a loose fibrovascular stroma.

The outer glands have a single layer of tall columnar epithelium with a supranuclear clear zone and a well-defined condensed luminal margin (Fig. 3). The epithelium of the inner glands is lower and sometimes has more than one layer of cells; the cytoplasm is more basophilic and homogeneous (Fig. 4). Both inner and outer glands are surrounded by a narrow band of plain muscle, and the whole organ lies in a loose fibrovascular stroma. The ducts of the prostate, seminal vesicles and vasa deferentia are lined by a low columnar epithelium in which there are occasional mucus-secreting cells particularly near the urethra. The utricle, which has a thin muscle coat, is lined by a single layer of basophilic, mucin-secreting epithelium, but its lower end and the vagina masculina are often lined by transitional epithelium.

Effects of age and treatment on the outer prostate

Secretion first appeared 4–6 weeks after birth, and the gland had its normal adult form by the age of 3–4 months. The secretion was stained strongly by the PAS reaction, but mucin was not normally present in secretion or epithelium.

In older animals epithelial changes appeared. Mucous metaplasia (Table II) was never seen in animals less than a year old, was present to some extent in about a third of the animals aged 2–3 years, and was widespread in almost all older animals. The columnar epithelium of the glands became lower, and globules of mucin appeared in the cytoplasm and later filled the lumina of the acini.

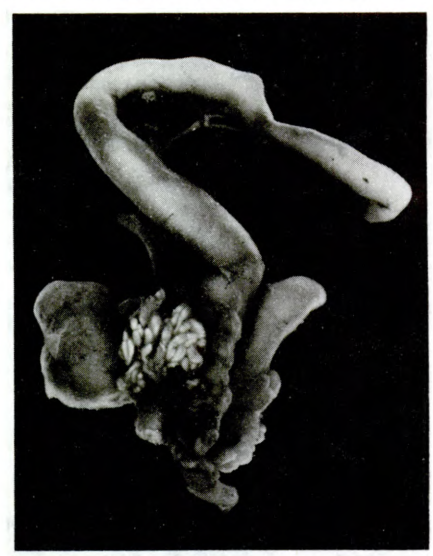

FIGURE 1. Lateral view of prostate complex from an adult guinea pig showing bladder (lower left), seminal vesicles and inner prostate, closely applied to the lower end of seminal vesicles. The smaller outer glands (white in the fixed specimen) lie on the inner glands. The upper portion of one seminal vesicle has been removed.

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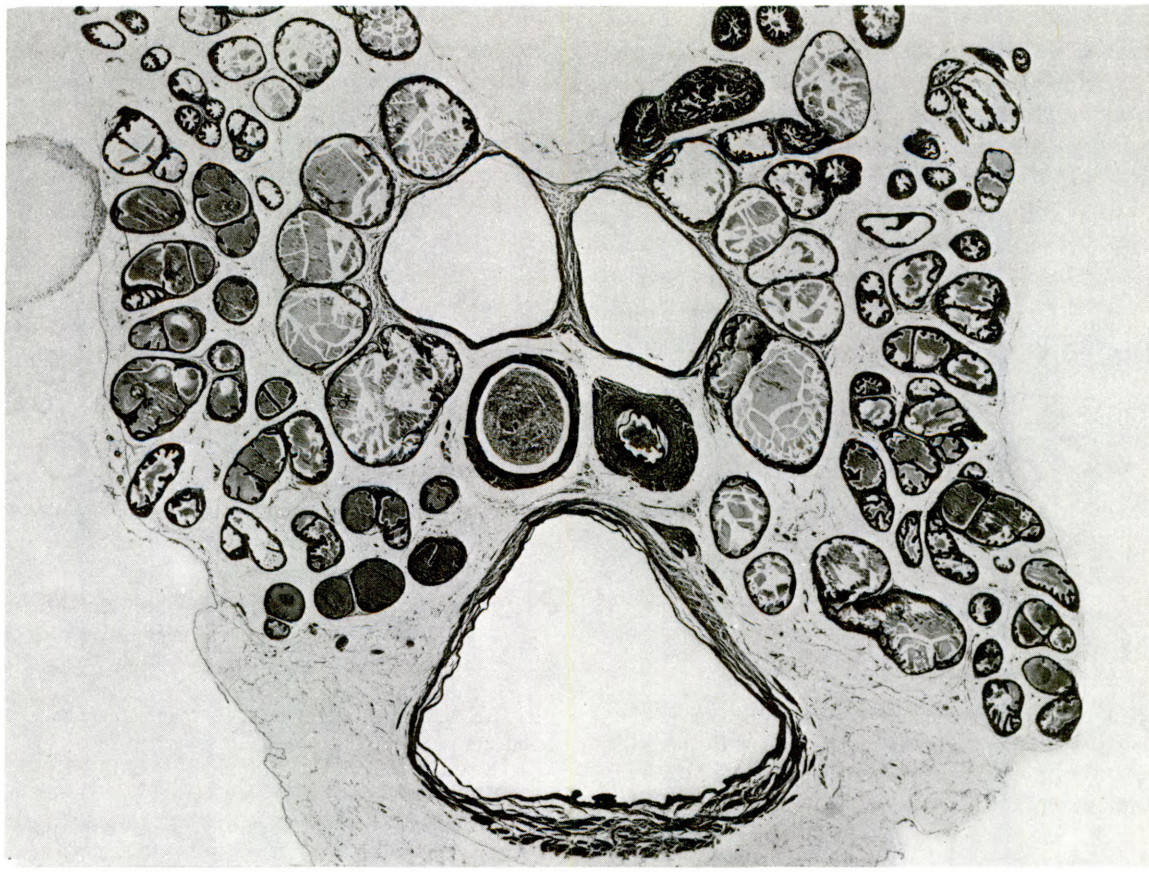

FIGURE 2. Transverse section through the prostate of an 8-month old guinea pig. The bladder (B), vasa deferentia and seminal vesicles (SV) are median. The inner coagulating glands are closely applied to the seminal vesicles, and the less distended glands of the outer prostate surround them. Hematoxylin and eosin. X 8.

This change was patchy at first but spread to involve most of the gland (Fig. 5).

The development of spindle-celled or squamous metaplasia followed a similar course (Table III). The normal single layer of columnar cells was replaced by several layers of flattened spindle cells, and in some cases there was true squamous metaplasia with keratin formation. The change sometimes affected only part of an acinus, and there was an abrupt change between the normal and metaplastic epithelium (Fig. 6). It was only seen in animals also showing mucous metaplasia although both types of metaplasia were not necessarily present in the same acini.

Effect of castration

The prostate remained small and undeveloped for some months in animals castrated within 2 weeks of

TABLE II—Incidence of Mucous Metaplasia in Outer Prostate

Group	Expt. No.	Age in years 0–1	1–2	2–3	3–4	4–5	over 5
N		0/39	6/17	9/29	19/22	8/8	3/3
C	1	1/12	0/2	1/3			
	2		0/2	4/13	3/4	4/5	
S	1	7/13	5/5		2/2		
	2		5/5	13/15	2/2		
CS	1	6/13	2/3	2/3			
	2		16/17	18/18		3/3	

Experiment numbers as in table 1.

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FIGURE 3. Normal epithelium of the prostate proper showing supranuclear clear zone and condensed free margin. Hematoxylin and eosin. X 500.

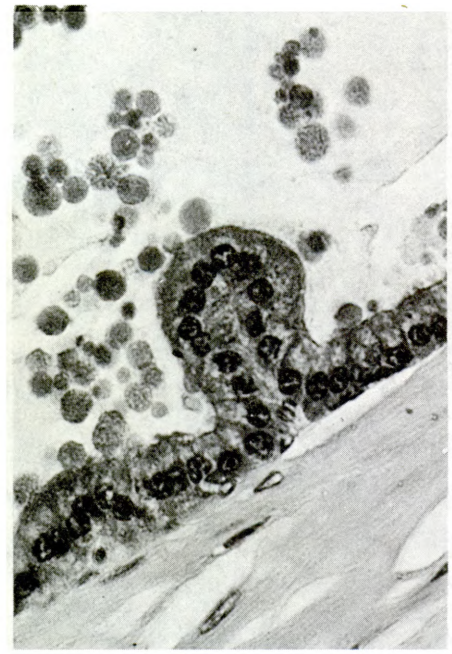

FIGURE 4. Normal epithelium of the coagulating gland showing globular secretion. Hematoxylin and eosin. X 500.

birth (Fig. 7), but even 6 weeks after operation some secretion was present and epithelial papillae were sometimes found. By 5 months the epithelial cells were taller, and some had well developed supranuclear clear zones. The gland remained in this state until at 12–14 months there was a gradual restoration of normal size and structure (Fig. 8). Castration in the older animals was followed by atrophy of the gland and loss of secretion. The epithelium became flattened, and the supranuclear clear zone was lost. Atrophy was complete 4 months after operation, and no secretion was then present. Secretion began to reappear after 11 months, and in the second year the gland resumed its normal size and appearance.

The epithelial cells were often tall columnar with ovoid nuclei sometimes forming intra-epithelial acini (Fig. 9). This hyperplastic change was very marked in one animal aged 59 months that had been castrated for 44 months and that also had a small adrenal cortical adenoma.

Mucous metaplasia appeared in the same way as in the normal animals. Epithelial stratification appeared in the older castrates, possibly less frequently than in intact animals, but squamous metaplasia was not observed.

TABLE III—Incidence of epithelial stratification in the prostate

Group	Expt. No.	Age in years 0–1	1–2	2–3	3–4	4–5	over 5
N		0/39	1/17	6/29	18/22	6/8	3/3
C	1	2/12	0/2	0/3			
	2	0/6	0/5	2/14	3/5	1/5	
S	1	0/13	1/5				
	2	0/10	1/10	2/18	0/2		
CS	1	0/13	0/3	0/3			
	2	0/9	8/23	6/18	2/2	3/3	

Experiment numbers as in table 1.

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FIGURE 5. Transverse section through the prostate of a 39 month old guinea pig showing mucoid secretion (black) in almost all glands of the outer prostate. PAS, alcian blue, orange G and hematoxylin. X 8.

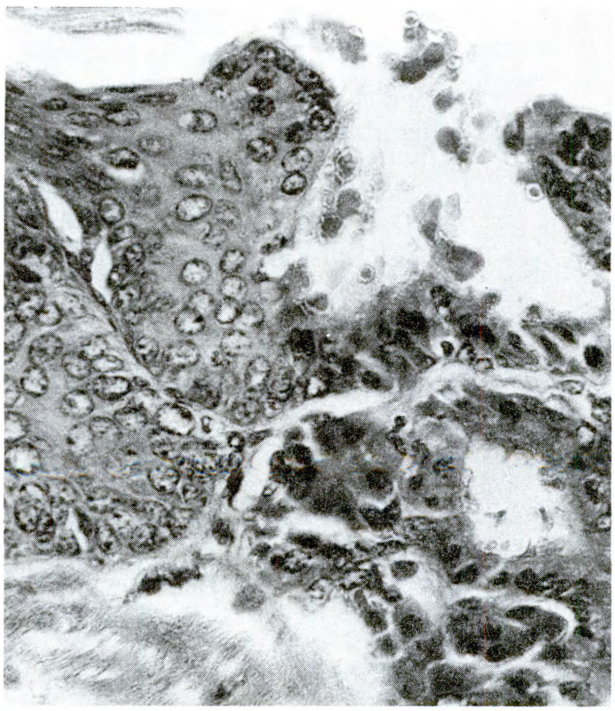

FIGURE 6. Abrupt transition between columnar and metaplastic squamous epithelium in the outer prostate of a 33 month old guinea pig. Hematoxylin and eosin. X 500.

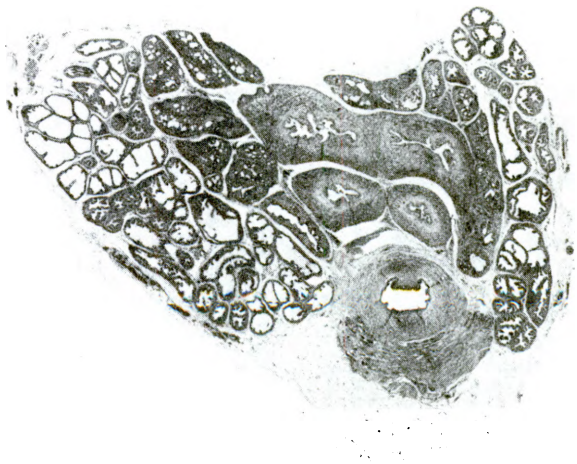

FIGURE 7. Transverse section through the prostate of a 3 month old guinea pig castrated within 2 weeks of birth. Hematoxylin and eosin. X 8.

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FIGURE 8. Transverse section through the prostate of a 16 month old guinea pig castrated within 2 weeks of birth, showing almost normal size of prostate and seminal vesicles. Hematoxylin and eosin. X 8.

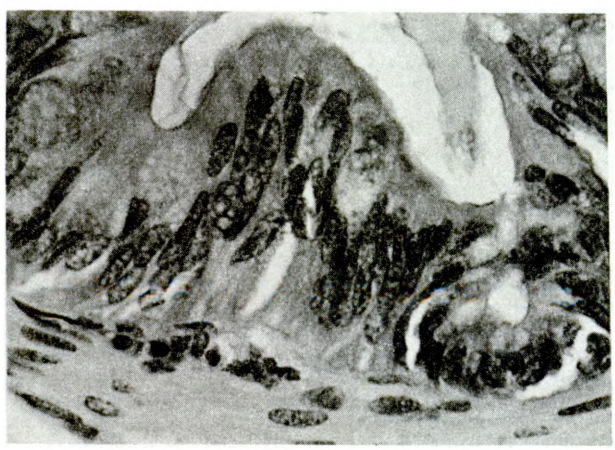

FIGURE 9. Hyperplastic epithelium of outer prostate of a 59 month old guinea pig castrated 15 months previously. Hematoxylin and eosin. X 500.

Effect of stilbestrol

The prostate remained small in the animals treated when young and became atrophic in those treated when adult (Fig. 10). The epithelium remained or became low, and the supranuclear zone did not develop or disappeared. There was some thickening of the muscle coat.

The mucoid change was frequent in all treated groups and appeared early (Table 2). It was present and often widespread in virtually all animals that had been treated for 8–12 months whatever the age at which treatment started. It tended to begin in the ventral parts of the gland (Fig. 11) and involved the dorsal area later. Stratification was not so commonly found.

Effect of combined treatment

Stilbestrol had much the same effect in castrates as it did in intact animals. The mucous metaplasia tended to appear rather earlier and was usually more widespread than in stilbestrol-treated intact animals.

Effects of age and treatment on the inner prostate (coagulating glands)

The mucous metaplasia and stratification seen in the epithelium of the prostate in ageing animals was never seen in the coagulating gland.

Effect of castration and stilbestrol

The changes produced in the coagulating gland were slower in onset and less extensive than in the outer prostate. After castration the epithelium tended to retain its structure, and secretion was usually present. However, there was some atrophy and as with the outer prostate this was reversed in the second year after operation. Mucous metaplasia and epithelial stratification were produced in the stilbestrol-treated animals but were much rarer than in the outer prostate.

Seminal vesicles

The seminal vesicles are lined by a single layer of

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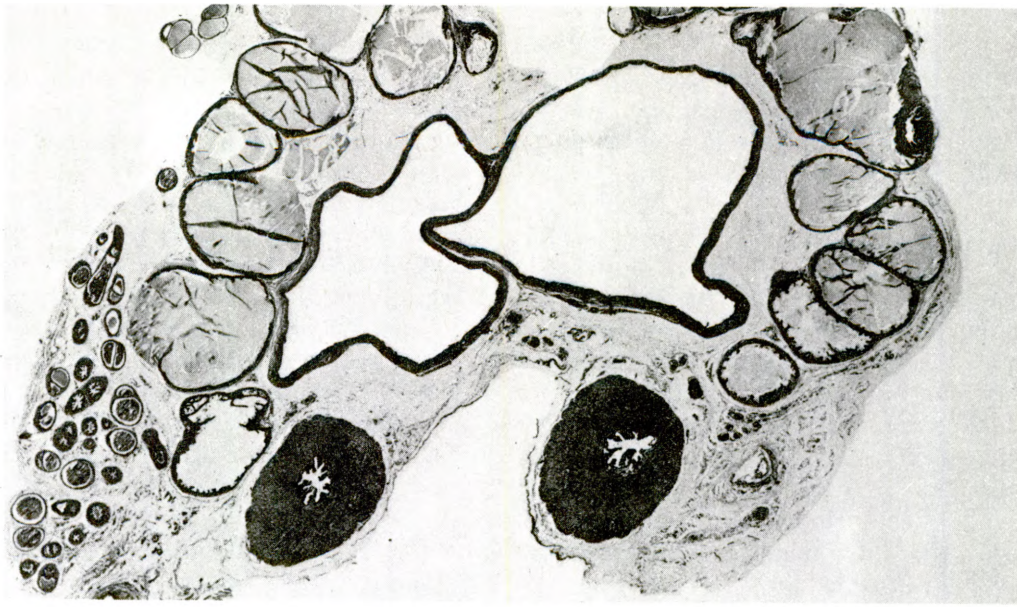

FIGURE 10. Transverse section through prostate of a 14 month old guinea pig treated with stilbestrol for 9 months. The outer prostate is missing from the section on one side but shows complete atrophy on the other side. The muscle coats of the vasa deferentia are greatly thickened. Hematoxylin and eosin. X 8.

FIGURE 11. Transverse section through prostate of a 29 month old guinea pig treated with stilbestrol for 11 months. Both coagulating glands and outer prostate are atrophic, and there is mucous metaplasia (black) of the ventral glands of the latter. Hematoxylin and eosin. X 8.

columnar cells which sometimes contain light brown pigment granules. This epithelium did not undergo mucous metaplasia nor stratification in older animals. As with the prostate, the effects of castration were temporary; the atrophy or failure to develop lasted several months, but after that the cells of the epithelium increased in height. By 16 months after operation there was little or no difference between the condition in the castrates and that in normal animals. The weight of the vesicles showed the same changes as the prostate though it tended to be more variable.

Stilbestrol treatment increased the number of mucin-secreting cells in the epithelium of the ducts. These cells became piled up to form epithelial plaques in which small cysts appeared, often containing mucin. This was followed, particularly in the immature animals, by squamous metaplasia, beginning at the lower ends of the ducts near the urethra. While the mucous change and formation of epithelial plaques spread a little way into the vesicles themselves, squamous metaplasia was confined to the ducts. These changes appeared earlier in the animals treated when immature than in those treated when older.

In the older animals treated with stilbestrol for more than a year, the seminal vesicles were often filled with pus (10 animals), and this was also seen in four of the younger animals.

In the animals treated for longer time the ducts

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were often dilated, probably owing to obstruction by the hyperplastic stroma (see below) that sometimes projected as an ulcerated polyp into the dilated ducts.

ASSOCIATED STRUCTURES

Utricle

This structure opens from a narrow groove on the verumontanum and consists of a narrow tube (the vagina masculinus) which becomes bicornuate (the uterus masculinus) cranially. This is lined by a single layer of basophilic foamy, mucin-secreting epithelium merging at its lower end and in the vagina masculinus into transitional epithelium. It has a thin muscle coat. None of the changes seen in the prostate of the older animals were found in the utricle, and castration had little effect on its structure.

Stilbestrol treatment, however, produced within one week an enlargement of the utricle which became filled with sheets of foamy mucin-containing cells with small pyknotic nuclei. The epithelium beneath these foamy cells was generally flattened although in some places it retained its normal columnar form. Later squamous metaplasia occurred, beginning in the vagina masculinus and then extending to involve much of the uterus masculinus.

This proliferation and metaplasia of the epithelium was associated with hyperplasia of the muscle and stroma of the verumontanum forming large tumour-like nodules (Fig. 12) containing, in varying amounts, muscle, fibro-vascular stroma and epithelium. The epithelial proliferation was sometimes considerable, and clumps of irregular small acini or metaplastic squamous cells infiltrated the muscle bundles. These infiltrating cells contained no mucin. Less frequently there were small adenomatous nodules with groups of tubules lined by apparently normal utricular epithelium.

These changes were more pronounced in the stilbestrol-treated castrates; the utricular tumours were larger, and squamous metaplasia was more common. The tumours were often cystic in the immature animals. In one animal in which no implant

FIGURE 12. Transverse section through prostate of 3½ month old guinea pig treated with stilbestrol for 3 months. There is a large fibroepithelial tumour in the utricular region of the verumontanum. The epithelium of utricle and vasa deferential are squamous. Hematoxylin and eosin. X 16.

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had been given for 6 months the utricle was cystic, but there was no proliferation of the epithelium—probably this had regressed.

Vasa deferentia

These ducts are lined by a low columnar epithelium which, like all those of the other structures as they pass through the urethral wall, contains scattered mucus-secreting cells. There was no particular change with age, but after castration there was an increase in subepithelial connective tissue and a striking subnuclear vacuolation of the lumenal layer of the epithelium and a flattening of the basal layer. These changes were prominent 4 months after operation, but the normal appearance was recovered after 9–12 months.

Stilbestrol treatment increased the number of mucous cells in the epithelium, and epithelial plaques like those in the ducts of the seminal vesicles appeared sooner and were more extensive. Vacuolation of the epithelial cells, like that in the castrates, was sometimes produced. There was also dilatation of the ducts in older animals as there was in the ducts of the seminal vesicles.

Stroma

The fibromuscular stroma of the verumontanum often contained small foci of lymphoid and plasma cells in the older animals, but no other changes were apparent. Stilbestrol treatment caused hyperplasia of the stroma which, as already described, led to the production of mixed tumours with the proliferating utricular epithelium. But often, particularly in the larger tumours, there was no epithelial component, and the nodules then resembled the fibromatous nodules described by Lipschutz (4) in other organs of estrogen-treated guinea pigs, though they often contained calcified areas and sometimes bone. Small collections of mucin, presumably derived from epithelium that had been destroyed, were also sometimes present.

Musculature

The muscle coat surrounding the prostate complex and the seminal vesicles became thickened in the stilbestrol-treated animals, and there was fusion of the muscle of the coagulating gland and seminal vesicles. The hyperplasia of the muscle in the verumontanum and its participation in the formation of tumours has already been described.

DISCUSSION

Androgenic activity after castration

Normal structure is restored to the prostate, seminal vesicles and coagulating glands several months after castration of immature or adult animals. The androgen that produces this effect presumably originates in the adrenal cortex, where a slow change in the pattern of steroid synthesis must occur.

Prostatic metaplasia

The remark that "the human prostate and the prostates of most experimental animals have individual anatomical peculiarities which make comparison fruitless in some cases, and difficult in all" (5) might equally apply to the metaplastic changes induced by excess estrogen in the prostate of different species. Most authors agree that metaplasia, usually squamous metaplasia, is common in the utricular region and may extend from the urethra along the prostatic ducts. But squamous metaplasia of the acinar tissue is found frequently only in the mouse and dog—certainly it is rare in man, monkey, rat, guinea pig, or rabbit (6,7).

Mosinger (8) refers to 'keratinisation et mucification' of the coagulating glands of guinea pigs treated in much the same way as ours, though we found mucous metaplasia to be confined to the outer prostate. The only other mentions of a mucous change are in the prostatic ducts of new born children (9) and in the terminal parts of the ejaculatory ducts of entellus monkeys treated with excess estrogen for a year (10). Possibly it occurs in other species but has been missed in preparations not specially stained.

It is tempting to relate the mucous change to the stimulation of adrenal cortical secretion produced by estrogen in the guinea pig (12). The situation might be analogous to that in the vaginal epithelium, where the squamous proliferation produced by estrogen is converted to mucification when other steroids, including corticosteroids, are given in addition. Lipschutz (2) proposed such an explanation of the vaginal mucification produced in spayed guinea pigs given small doses of oestrogen.

The occurrence of mucous metaplasia in the prostate of aging guinea pigs is difficult to understand, but nothing is known about estrogen production in the male guinea pig. Mucin is rarely seen in the outer glands of the human prostate even in elderly men although it does occur in the cells and acini of

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many well-differentiated prostatic cancers (13). Stilbestrol treatment in man causes a decline in endogenous estrogen and androgen production like that seen normally in middle and old age (14). This could be taken as another instance of stilbestrol accelerating or reproducing a normal ageing process. Until more is known about the changes in adrenal-cortical function after castration or estrogen treatment and during normal ageing in the guinea pig, little more can be said.

Exact measurement of hormone levels in treated animals, as may now be possible, may help in the understanding of the process. In preliminary experiments in which small groups of animals were treated with stilboestrol 3 months after castration the changes developed more rapidly. This suggests that the state of the cells at the time of treatment may influence the degree of structural change.

Changes in the utricle

The Mullerian derivative would be expected to respond rapidly to excess estrogen as we have found. Again the mucous change may be associated with increased corticosteroid production. The fibromuscular epithelial tumours produced in this region resemble those described by Lipschutz and his colleagues (4) and by Mosinger (8).

The nodules are not analogous to those seen in benign nodular hyperplasia of the prostate in man. This arises in the inner group of prostatic glands and rarely involves the utricle (15). Utricular hyperplasia is very uncommon in man even after prolonged excessive estrogen treatment (Franks, unpublished).

Recovery after castration

The observation of Spiegel (16) that the atrophy of the prostate occurring after castration of 3 young guinea pigs is not permanent is confirmed by our own observations. The age at which the animals are castrated does not seem to affect the situation. Again, secretion of adrenal steroids is the probable explanation—in this adrenal androgen is implicated. This would suggest that the guinea pig adrenal has a greater capacity for androgen secretion than the rat (17,18). The recovery of structure was particularly marked in one animal with an adrenal-cortical adenoma, but such tumours are rarer in castrated guinea pigs than in castrated mice (19). Certainly the castrates continue to excrete large amounts of 17-oxosteroid in the urine (12).

The atrophy that follows castration develops more slowly than in other species, but although the epithelial cells in some acini retain their normal structure for some time, their ability to produce normal secretion may be lost earlier (20). We cannot say whether the structural recovery of the gland is paralleled by a recovery of full secretory function.

The prostate of the stilbestrol-treated animals, whether intact or castrated, remains atrophic which suggests that the stimulation of adrenal-cortical secretion mainly affects the non-androgenic corticosteroids.

SUMMARY

Immature and adult male guinea pigs were castrated and/or treated with stilbestrol for periods of up to 4 years. Stilbestrol was given by the implantation of subcutaneous tablets from which the absorption was about 100μg daily.

The atrophy of the prostate, coagulating gland and seminal vesicles following castration is not permanent. During the second year after operation, all these structures recovered their normal weight and histological structure.

Mucous metaplasia and stratification of the prostatic epithelium began to appear in normal animals that were 1–2 years old and were present in almost all animals more than 3 years old. Similar epithelial changes occurred within a year of starting stilbestrol treatment which in this way mimics or accelerates anormal ageing change. There was no recovery from the general atrophy of the gland produced by stilbestrol unless treatment was discontinued.

Similar epithelial changes were seen in the ducts of the coagulating glands and seminal vesicles but rarely extended into the glands.

Fibromuscular hyperplasia of the stroma occurred in stilbestrol-treated animals in which fibro-myoepithelial tumours of the utricle and stroma of the verumontanum were often produced.

CONCLUSIONS

Although the treatment used in these experiments did not produce lesions resembling those of benign prostatic hyperplasia, the epithelial changes in the guinea pig resemble more closely those that occur in the ageing human prostate. This suggests that the guinea pig may be a more useful animal than other rodents for a study of the direct effects of hormones on the prostatic epithelium, particularly if dose levels approaching the physiological are used. As in almost all species, a true prostatic

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hyperplasia did not occur. The possibility that the growth stimulation may be due to non-steroidal stimuli must be considered. Although this may be pituitary in origin it is also possible that some specifically human environmental agent may provide a necessary cofactor.

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DISCUSSION

J. E. McNeal: This is just a brief look at changes in ducts that pass by the edges of BPH nodules. The phenomenon I'm interested in is that the duct on its inner side nearest to the nodule gives a series of branches that appear to be pulled into and proliferate into the nodule, while on the outer side of that same duct there is no such proliferation. Ducts often show epithelium of increased height on the inner side facing the nodule, while the outer side appears somewhat atrophic. This morphologic feature suggests to me that there is something on the inner side, perhaps a diffusible inducer, which is causing hypertrophy and hyperplasia of the epithelial cells. More significant is the production of new architecture that is selectively oriented in the central focus of the nodule. I think this is the way in which nodules get started, at least those nodules that grow to considerable size. So, I'd like to add a third viewpoint to those of the urologist and the biochemist. The urologist looks on BPH as something that causes people urethral obstruction. The biochemists here tend to look at whatever they can do to make a prostate grow larger. I feel it is more important to understand the mechanism of this peculiar inductive effect. If this type of induction could be produced experimentally, even if it didn't make a big gland, and even if it didn't make urethral obstruction, I think we would be closer to the real pathogenesis of the human disease.

L. N. Franks: Could I just make one last comment? Walsh mentioned the Scotish terriers. I think

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it is worth pointing our that this is also a breed that does get a high incidence of sertoli cell tumors.

G. WILLIAMS-ASHMAN: I'd like to ask Dr. Franks whether or not the tissue that he showed us was the coagulating gland or the ampullary gland? There's some confusion with regard to the cystic dilation that occurred in an inbred strain of hamsters.

FRANKS: The hamster has an ampullary gland between the bladder and the seminal vesicle, and the guinea pig, and probably the mouse, has it too. In some rodents it is actually inside the muscle sphincter so that it can barely be seen. In others, such as the hamster it projects as a separate gland. This gland sometimes does become cystic, particularly if you treat it with estrogen. In mice, cystic changes sometimes occur in the utricle with enormous distention and fluid filled with polymorphs and sometimes distention in the ampullary gland. But the differences between rodents is really just anatomical; the glands are there, but they may be inside or outside the muscle.

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HUMAN BENIGN PROSTATIC HYPERPLASIA IN CELL AND ORGAN CULTURE

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Cell and organ culture have added a new approach to the study of hormonal effects on human prostatic hyperplasia. In animal experiments the final interpretation of results is complicated by many systemic influences, such as the nutritional, immunological and in particular the hormonal status of the animal. In in vitro systems these factors are eliminated, and the direct effect of hormones can be studied on the cellular level under well controlled experimental conditions. The system also allows a strict dosage control of hormones and an easy replication of experiments which makes it possible to obtain both qualitative and quantitative data.

Tissue culture falls into two main categories: the culture of cells and the culture of organs. The former consists of cells migrating from and surrounding explant cultures or more frequently of cells grown in monolayer or suspension. In organ culture the various tissue components, their anatomical relationship and function are, under suitable conditions, preserved in vitro.

Much basic information relating to the effects of androgens and antiandrogens and their mechanism of action has been obtained using organ cultures of rat prostate glands as experimental model. These organs remain androgen dependent and responsive in vitro; if explanted into androgen-free medium the epithelium regresses as in the organ following castration while addition of testosterone prevents the regressive changes (1). Like the organ in vivo (2) the isolated gland in culture converts testosterone to various 5α metabolites, principally dihydrotestosterone which in turn is converted to 3α and 3β-androstanediol (3). Exposure of the glands to these metabolites revealed that dihydrotestosterone is a potent androgen, involved in cellular differentiation as well as cell renewal while 3β androstanediol stimulates secretory activity (4).

Human benign prostatic hyperplasia shows a similar pattern of testosterone metabolism as the rat prostate (5), and the results obtained in the rodent gland provide a relevant and valuable background for hormonal studies of the human tissue. The basic aim of such studies has been to establish whether the growth and maintenance of the tissue depend on androgens and can be modified by steroid hormones and to relate hormonal response in vitro to that of the patient. The cultivation of human benign prostatic hyperplasia has been approached by establishment of the tissue in cell culture and in organ culture.

Cell Culture

Using a biological medium and Carrel flasks, Wajewski & Przevorska-Kaniewich (6) set up a large number of explants from benign prostatic hyperplasia of which half became surrounded by fibroblast-like cells. Testosterone and stilbestrol inhibited the outgrowth (Table I).

Fraley, Ecker & Vincent (7) developed a cell line from a prostatic adenoma. The tissue of origin showed marked glandular hyperplasia with some areas of squamous metaplasia. The cells grew well in monolayer cultures without androgens in Eagle's minimum essential medium (MEM) supplemented with glutamine and bovine serum. They were des-

*Sir Halley Stewart Fellow.

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TABLE I—Cell Culture of BPH

Authors	Method	Cell type	Results
Wajewski & Przeworska-Kaniewich (1965) (6)	Explant culture	Fibroblastic	Normal growth without hormones; inhibition by testosterone or stilbestrol.
Fraley, Ecker & Vincent (1970) (7)	Cell line MA 160 in monolayer	Epithelial	Normal growth without hormones.
Lasnitzki (1975) (11)	MA 160	Epithelial	Normal growth without hormones; inhibition by pharmacological doses, stimulation by physiological doses of testosterone, dihydrotestosterone or estradiol.
Brehmer, Marquardt & Madsen (1972) (15)	Short term cell culture in monolayer	Epithelial and fibroblastic	Normal growth without hormones; inhibition by testosterone, estrone or diethyl-stilbestrol.

ignated MA 160 and have now been carried through more than 500 passages. The cells are epitheloid, and their fine structure reveals rough endoplasmic reticulum and microvilli typical of secretory cells.

Normally, human adult cells have a finite life span in culture. The long survival of MA 160 in vitro suggests that the cells have undergone spontaneous transformation. In fact, the cells exhibit many features characteristic of this process: rapid growth, heteroploidy, irregular morphology and the ability to form tumours on injection into the cheek pouches of immuno-suppressed hamsters (8). Cells from late passages grow also in non-immuno-suppressed hamsters and induce a high incidence of tumours (9).

The increased growth potential of MA 160 at later passages is accompanied by a change in testosterone metabolism. Ofner (10) compared the metabolism of testosterone of cells from the 12th and the 279th passages. In both, the conversion of testosterone followed a reductive pathway, but less dihydrotestosterone was formed by the cells from the later passage.

Attempts in this laboratory to reverse the anaplastic growth pattern and induce structural differentiation by a variety of hormones, including testosterone, its 5α metabolites and estradiol proved unsuccessful. On the other hand, the rate of growth was influenced by addition of hormones to the culture medium (11). Cell proliferation measured by the increase in cell number relative to that of the controls, was inhibited by pharmacological doses of testosterone, 3β-androstanediol and estradiol while dihydrotestosterone produced only a slight growth inhibition. Physiological concentrations of testosterone and estradiol alike raised the cell number slightly over that of the controls: dihydrotestosterone was more active and at the same dose nearly doubled it (Fig. 1).

It has been claimed (12,13) that many heteroploid cell lines including MA 160 have been contaminated with HeLa cells. These are derived from a cervical carcinoma of a black patient and have been propagated in vitro for over 20 years (14). The claim is based mainly on the presence of a rapidly moving (A) isozyme of glucose-6-phosphate dehydrogenose and chromosome markers characteristic for HeLa in MA 160 and other cell lines. This possibility cannot be lightly dismissed, but there are some features that do not fit HeLa. The fine structure of MA 160 is characteristic for a secretory cell, and unlike HeLa some tumours derived from MA 160 show pseudoglandular structures. Further it is not known whether HeLa cells metabolize testosterone, and a comparison of testosterone metabolism of MA 160 and HeLa should provide more decisive evidence for or against the theory of contamination. Even if not contaminated it is doubtful whether the transformed cell line is still comparable to the original tissue, and short term cultures of human prostate would be preferable to relate hormonal response in vitro to that of the patient.

Brehmer, Marquardt & Madsen (15) established cell cultures from human benign prostatic hyperplasia in monolayer which remained viable for 2-3 months. The cultures consisted of several cell types, polygonal or rounded cells and spindle shaped fibroblast-like elements, and grew well in the absence of androgens. Using Puck's method, (16) the authors expressed growth in terms of plating efficiency, i.e. the percentage of colonies developed from the seeding of single cells. Testosterone (Fig. 2), estrone and stilbestrol reduced the

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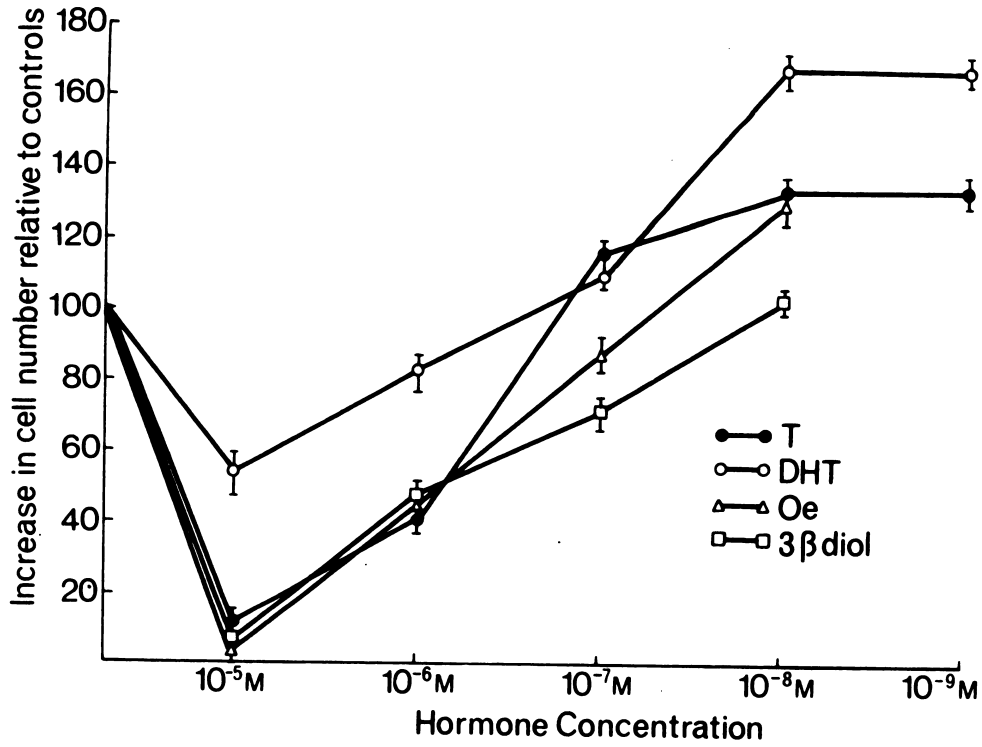

FIGURE 1. The effect of testosterone (T), dihydrotestosterone (DHT), 3β-androstanediol (3β-diol) and estradiol-17β (Oe) on the growth of MA 160 expressed as increase or decrease in cell number relative to that of the controls. (Lasnitzki).

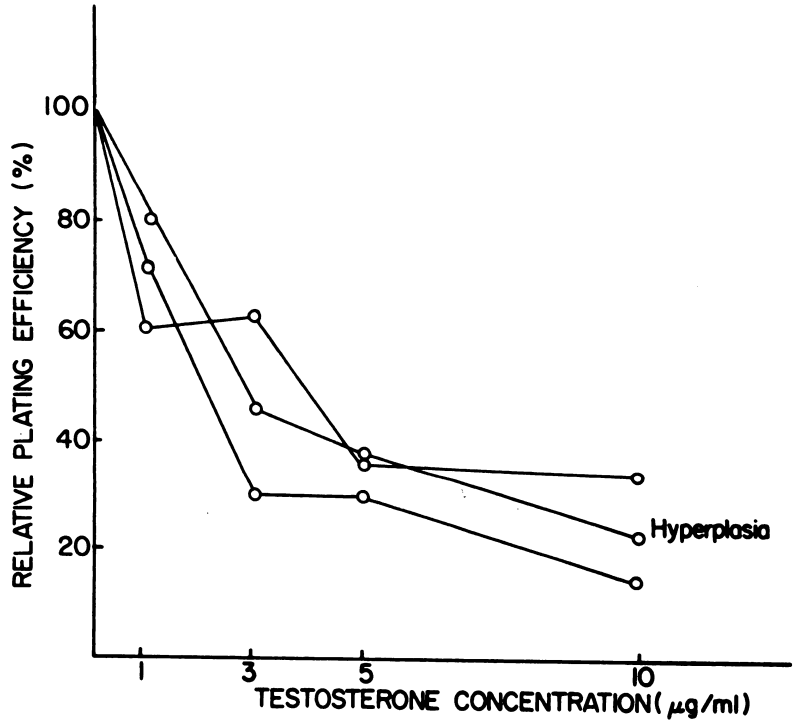

FIGURE 2. The effect of testosterone on the plating efficiency (percentage of colonies developed from the seeding of single cells) of cells derived from human benign prostatic hyperplasia obtained from three patients. (By courtesy of Brehmer, Marquardt & Madsen, 1972).

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plating efficiency progressively with dose, and stilbestrol proved to be a more efficient growth inhibitor than estrone (Table II).

Thus, interestingly, in both, short term cell cultures and MA 160 cellular growth seems independent of androgens and is inhibited by testosterone, estradiol and estrone at pharmacological concentrations. This result raises some doubt as to whether short term cell cultures are representative of the original adenoma. It must be remembered that at least 2–3 weeks elapse between the removal of the tumour and the establishment of the isolated cells in monolayer. Even during this short period a selection of cells best adapted to *in vitro* conditions takes place, and these may no longer be closely comparable to the more heterogenous cell population of the tumour. Moreover, stromal elements are an essential part of each tumour. According to Franks, Riddle, Carbonell & Gey (17) epithelium and stroma form a functional unit, and the expression of hormonal action depends on an intact epithelial-stromal relationship.

In organ culture these two components and their anatomical relationship are, under suitable conditions, preserved in vitro. In addition, the system avoids the disadvantage of time lag and selection. Fragments of adenomas can be explanted and exposed to hormones shortly after removal from the patient. The action of hormones can be examined on cell proliferation as well as cell differentiation and the effects on epithelium and stroma can be studied separately. In consequence, in organ culture the criteria of hormone action include maintenance of epithelial height and fine structure and of secretory activity.

TABLE II—Effects of Steroids on Plating Efficiency of Cells from Hyperplastic Prostates

Compounds	Concentration (μg/ml)	Plating Efficiency a	b	c
Estrone	-0.5	100	83	100
	1.0	100	68	100
	3.0	75	50	93
	5.0	58	43	77
	10.0	12	16	31
	20.0	0	0	
Diethyl-stilboestrol	0.5	100	100	100
	1.0	86	89	96
	3.0	50	66	69
	5.0	41	45	46
	10.0	0	10	0
	20.0	0		

By courtesy of Brehmer, Marquardt & Madsen, (1972)

Organ Culture

Schrodt & Foreman (18) (Table III) were the first to explant prostatic adenomas in organ culture. The tissue was grown in Trowell medium by a modified Trowell technique (19) on agar rafts supported on grids of tantalum mesh. In most explants the morphology of the epithelium and stroma were well maintained during the first three days and in some explants up to nine days. In older cultures the secretory epithelium was often low columnar and occasionally underwent squamous metaplasia. The fine structure of the epithelium in culture resembled that described by Brandes, Kircheim & Scott (20) for the fresh tissue, including abundant rough endoplasmic reticulum and secretory vesicles and granules. In cells showing squamous changes, bundles of intracytoplasmic filaments resembling those of tonofibrils were observed. Exposure to testosterone propionate caused severe epithelial necrosis.

Using a similar technique, MacMahon & Thomas (21) studied the effect of testosterone and stibestrol diphosphate on the maintenance of benign prostatic hyperplasia. The tissue was explanted in Eagle's basal medium supplemented with insulin and bovine serum. After one week in control medium, most explants showed well preserved alveoli embedded in fibro-muscular stroma. Some alveoli were lined with columnar, and others were lined with low cuboidal cells, and in some areas the secretory cells had undergone squamous transformation. Addition of testosterone or of stilbestrol diphosphate did not alter the morphology of the epithelium or stroma. Harbitz, Falkanger & Sander (22) extended the study to several other hormones and examined the effects of testosterone, dihydrotestosterone, estradiol-17β, progesterone and cyproterone acetate on human BPH grown in Trowell medium. They also found that the epithelium and its enzymes were well maintained in androgen free medium and further that none of the hormones altered epithelial morphology, the normal enzyme pattern, or DNA synthesis. In contrast, McRae, Ghanadian, Fotherby & Chisholm (23) reported that DNA synthesis was slightly increased in testosterone treated explants of BPH while glucose utilization was unchanged.

In androgen deprived target tissues one of the first consequences of testosterone treatment is an increase in RNA synthesis that precedes the increase in DNA synthesis and the restoration of normal morphology (24). RNA synthesis seemed, therefore, a sensitive criterion of hormonal effects, and Lasnitzki, Whitaker & Withycombe (25) at-

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TABLE III—Organ Culture of BPH

Authors	Source	Method	Results
Schrodt & Foreman (1971) (18)	Retropubic resection	Modified Trowell technique	Structural maintenance without androgens, necrosis after addition of testosterone propionate.
MacMahon & Thomas (1973) (21)	T.U.R.	Modified Trowell technique	Structure of epithelium maintained without androgens. Testosterone or stilbestrol do not alter morphology.
Harbitz, Falkanger & Sander (1974) (22)	Open prostatectomy	Trowell technique	Maintenance of structure and normal enzyme pattern without androgens. Testosterone, dihydrotestosterone, estradiol, progesterone and cyproterone do not alter morphology, enzyme pattern or DNA synthesis.
McRae et al. (1972) (23)	Open prostatectomy	Leighton tubes	Testosterone raises DNA synthesis slightly, does not affect glucose utilisation.
Lasnitzki (1975) (25)	T.U.R.	Modified Trowell technique	Increase of RNA synthesis in epithelium and muscle by testosterone, dihydrotestosterone, decrease by estradiol.

tempted to correlate the action of testosterone, dihydrotestosterone, 3β-androstanediol and estradiol-17β on epithelial structure with that on RNA synthesis. In non-supplemented medium and in the presence of hormones the epithelium multiplied to form several layers projecting into the lumen. In the controls the cells showed some evidence of squamous metaplasia. Testosterone and dihydrotestosterone prevented the latter and preserved the secretory character of the epithelium; in addition, dihydrotestosterone increased the proliferation of the epithelium beyond that seen in control and in the other experimental explants. In contrast to the androgens estradiol-17β caused much cellular breakdown including the loss of the secretory cells lining the alveolar lumen.

The cytological changes were reflected in variations of RNA synthesis. The incorporation of [^{3}H]-uridine into RNA was determined by autoradiography separately in epithelium and smooth muscle (Figs. 3–6) and expressed as the percentage of labelled cells and the average number of grains per cell. In the epithelium the uptake was increased by testosterone and dihydrotestosterone, reduced by estradiol, and not affected by 3β-androstanediol (Fig. 7). In the smooth muscle the number of labelled cells was similar in control and androgen treated explants and decreased by estradiol, but the grain counts per cell were raised by the androgens and again reduced by estradiol (Fig. 8). Thus, although the growth of the tissue in organ culture appeared independent of androgens, the explants continued to exhibit hormone responsiveness under the conditions of these experiments.

In conclusion, this brief review shows that under suitable conditions satisfactory growth or maintenance of human benign prostatic hyperplasia can be achieved in vitro. Although the tissue was derived from different patients and cultivated by different techniques the findings reveal nevertheless a generally consistent pattern.

In contrast to the rodent prostate, cells or tissues from benign prostatic hyperplasia grow or are relatively well maintained in androgen-free medium. It is unlikely that this property has been acquired during cell transformation or cell selection or is due to a loss of receptors since it occurs in the long term line MA 160, in short term cell cultures and in organ cultures explanted shortly after removal from the patient.

The retention of receptors is also indicated by the fact that growth can still be influenced by application of steroid hormones. At pharmacological concentrations, testosterone inhibits cell growth both in MA 160 and the short term cell cultures; at physiological or near physiological concentrations the hormone stimulates cellular proliferation in the cell line, MA 160. Surprisingly, estrogen and stilbestrol produce effects similar to those of testosterone and severely suppress cell growth at pharmacological concentrations.

The hormonal study of BPH in organ culture is based not solely on cell growth but includes maintenance of epithelium and its function as important criteria of hormonal action. While the data relating to androgen independence of growth and epithelial maintenance are in good agreement, those concerned with hormonal response of BPH in organ

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FIGURES 3–6. Autoradiographs of human benign prostatic hyperplasia labelled with [^{3}H]-uridine after 4 days' growth in organ culture. (Lasnitzki et al., 1975).

FIGURE 3. Alveolar epithelium in explant grown in non-supplemented control medium showing a fair number of labelled cells. x 690.

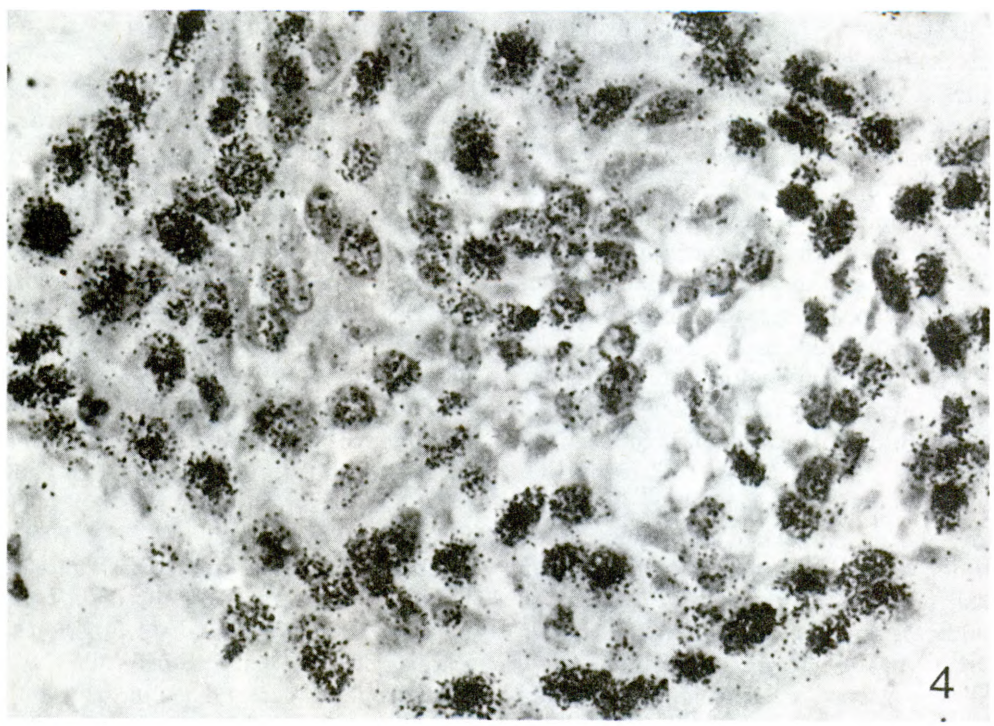

FIGURE 4. Alveolar epithelium in explant grown with testosterone (3 μg/ml) showing more and heavily labelled cells. x 690.

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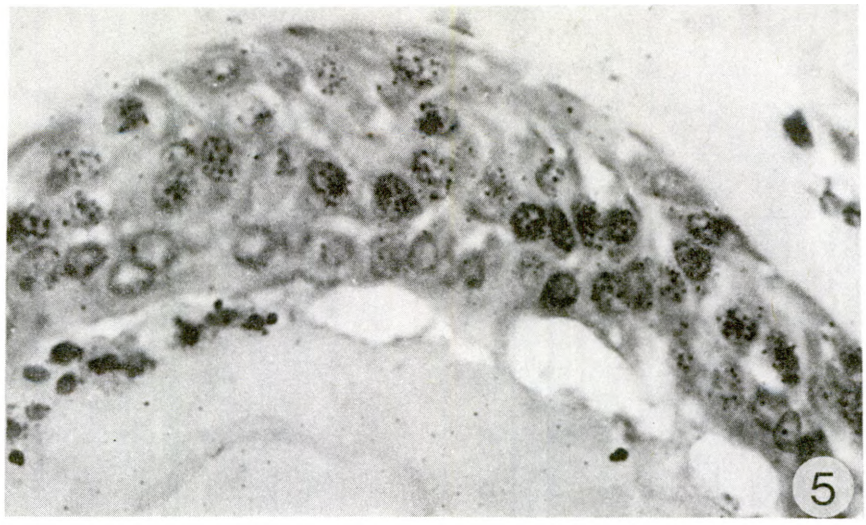

FIGURE 5. Alveolar epithelium in explant grown with estradiol-17β (3 μg/ml) showing fewer and weakly labelled cells. x 690.

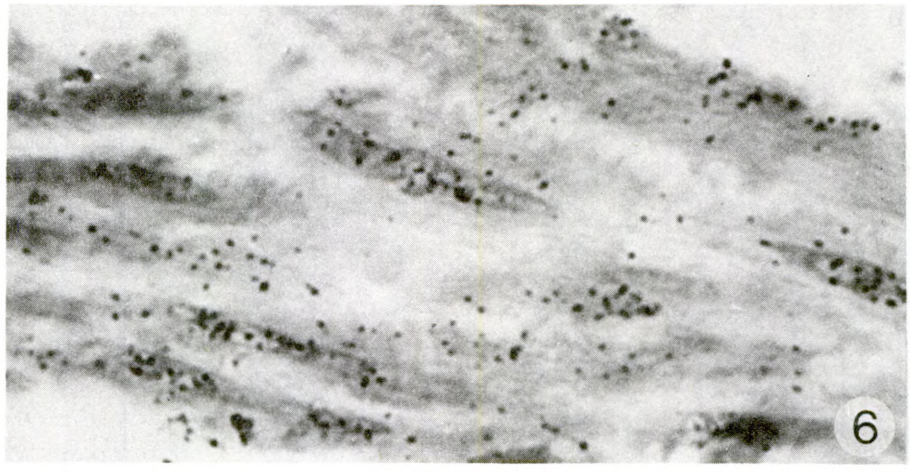

FIGURE 6. Labelled cells of the smooth muscle in explant grown with testosterone (3 μg/ml). x 1000.

culture are still controversial. MacMahon & Thomas (21) and Harbitz et al., (22) reported a failure of steroid hormones to modify the morphology of the epithelium or its enzymes. In contrast, alteration of epithelial growth or morphology and changes in RNA synthesis by testosterone, dihydrotestosterone and estradiol were demonstrated by Lasnitzki et al., (25). In this context, two points deserve special mention. First, unlike dihydrotestosterone which seems to play a similar role in the human tissue as in the rat prostate, 3β-androstanediol is ineffective and may, therefore, have no function in the maintenance of the human hyperplastic epithelium. Second, the changes in RNA synthesis induced by the steroid hormones in the smooth muscle cells suggest that, like the epithelium, they are also hormone responsive. Since the muscle forms a substantial part of the stroma the finding is important and should be considered in the evaluation of hormonal effects and their application to clinical therapy.

The hormonal studies on human benign prostatic hyperplasia are still at an early stage and confined to a relatively small number of investigators. Many more observations are needed to resolve some of the contradictions inherent in the results obtained so far and to relate growth and hormonal response of the tissue in vitro to that of the individual pa-

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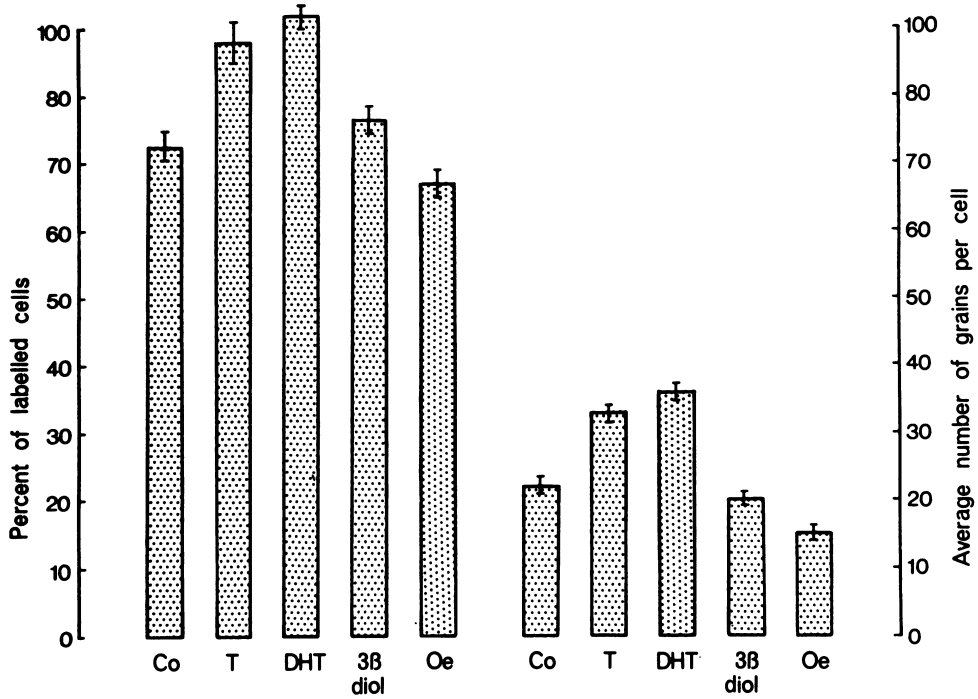

FIGURE 7. Incorporation of [^{3}H]-uridine in epithelium of human benign prostatic hyperplasia grown for 4 days in non-supplemented control medium (Co) or treated with testosterone (T), dihydrotestosterone (DHT), 3β-androstanediol (3β-diol) and estradiol-17β (Oe). The values are expressed as the percentage of labelled cells and their standard variation (left side of histogram) and average number of grains per cells and their standard variation (right side of histogram). (Lasnitzki et al., 1975).

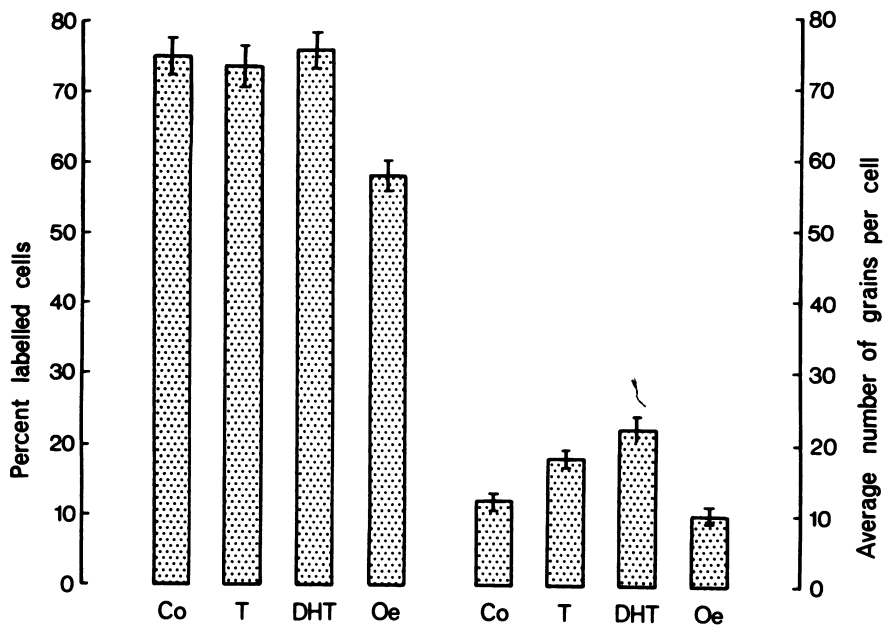

FIGURE 8. Incorporation of [^{3}H]-uridine in smooth muscle cells of human benign prostatic hyperplasia grown for 4 days in nonsupplemented medium (Co), or treated with testosterone (T), dihydrotestosterone (DHT) and estradiol-17β (Oe). The values are expressed in the same way as in Fig. 7. (Lasnitzki et al., 1975).

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tients. Since some of the differences may be due to variations in culture techniques, it would be invaluable to standardize the experimental procedures. To simulate the more complex conditions in vivo it would also be important to examine the effects of a wide range of hormone concentrations and to investigate the interaction of androgens with other hormones relevant for prostatic growth and maintenance. It is hoped that this report will stimulate experiments on these or similar lines.

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DISCUSSION

P. OFNER: I would like to supplement Dr. Lasnitzki's material by describing evidence for maintenance of canine prostate in organ culture. The prostate of a 3 year old Shepherd dog weighed 35 grams. The prostatic explants before culture showed marked hyperplasia, and most of the morphologic features of the explants are maintained in Trowell's T8 medium containing 1 μM testosterone without serum supplement after 1 and 3 days in

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culture. I am indebted to my colleague Dr. Irwin Leav for the histology. ^{3}H-testosterone (10 nM) penetrated into epithelial cytoplasm and nuclei. This work was carried out in collaboration with Dr. Walter Stumpf using dry-mount autoradiography.

F. H. SCHROEDER: I would like to mention one thing about Dr. Lasnitzki's work. We have attempted to establish the hormone dependence of MA-160 cells. Using an androgen free medium with charcoal extracted or dichloromethane extracted serum, MA-160 cells grow just as well without any testosterone in the medium as they do with testosterone.

D. F. PAULSON: The question has recently been raised as to the true origin of MA-160 and the nature of the cell type involved. I think it would be of value to karotype these cells to make sure you knew exactly what you had.

I. LASNITZKI: You refer to the possibility that the cell line has been contaminated with HeLa. The chance of contamination should be very low as neither Microbiological Associates from which our MA-160 was obtained nor my own laboratory ever carried HeLa cells.

P. OFNER: The epiethelial origin of the MA-160 cells has been questioned because no specific biochemical markers could be found to identify the cells. We have published a preliminary report on the pathways of ^{14}C-testosterone and ^{14}C-5α-dihydrotestosterone metabolism by low and high number passages of the MA-160 line (Ofner et al., In Vitro, *8*: 436, 1973). The cells were obtained directly from Vincent. Cells of low and high passage numbers metabolized both radiosteroids predominantly by the 17β-hydroxysteroid pathway as would be expected for prostatic epithelial cells. Last year Nelson-Pees et al. (Science *184*: 1093, 1974) described marker chromosomes characteristic of HeLa in MA-160 passage No. 106. We are performing a control study of androgen metabolism by several HeLa lines before publishing a detailed account of our findings. HeLa derived from human cervical adenocarcinoma would be expected to metabolize testosterone predominantly by the oxidative pathway. Mr. Vincent assures me that there was no source of HeLa cells in his laboratory at the time the MA-160 line was established.

M. VINCENT: I would like to make two corrections. First, MA-160 was started at both the Navy Tissue Bank and in my laboratory. Since I was the donor of the tissue, I was unable to process the tissue at the time of surgery. Commander Perry at the Navy Tissue Bank agreed to initiate the culture and take care of it until I was able to get back to work. I outlined the experimental design for explanting, and when I returned to my lab, I took possession of all existing cultures that were in the second passage. Second, no MA-160 cells that ever left my laboratory, whether they were sent to Fraley and Ecker or to any other investigators, were ever brought back into my laboratory. As a point of information, the low and high passage reference stocks (ranging from passage 5 to passage 583) are under my control, cryopreserved and stored in the vapor phase of liquid nitrogen. This reference stock is PPLO-Free.

PAULSON: That's excellent information because there are multiple rumors about MA-160.

VINCENT: The complete history and characterization of MA-160 is being prepared for publication, and all of the details will be presented.

R. KAHN: Fischer, Burkel, and I have been cultivating dog and human prostate for about a year and a half. Using needle biopsies of human prostate, we can obtain true peripheral prostate rather than tissue located just around the urethra. Some of the things you are seeing may be reflections of the cell types you started with. McNeal defined two distinct zones in man—a "central" periurethral zone and a "peripheral" zone. (Cancer 23:24–34, 1969).

L. FRANKS: In vitro techniques using human prostatic tissue have not been used to any great extent (1,2,3), yet the methods involved are not difficult.

Organ cultures of human prostate about 1.5cm^2 or more can be prepared using a simple slicing technique, with an ordinary safety razor blade. The limiting factor is not the surface area but the thickness, which must be less than 0.1cm, to allow free diffusion of oxygen. Epithelium, muscle and stroma can be maintained for some weeks, but there is sometimes a tendency to squamous or mucinous metaplasia (Figs. 1, 2 & 3). The great advantage of a system using large pieces is that it avoids the post-traumatic regenerative changes which occur in sub-lethally damaged epithelium (4).

Cell cultures can also be prepared, but we find that explants give better results than cell suspensions prepared by trypsinization. As with most cultures prepared from normal tissues there is a mixed mesenchymal and epithelial outgrowth. Epithelial cells can be maintained in primary culture for some weeks but are usually lost on transfer. Occasional cultures can be maintained for up to 48 months (5). Irregular giant cells often appear in these cultures shortly before they die out (Fig. 4).

A third technique, mechanical separation of epithelial and stromal cells, (5) can be used to inves-

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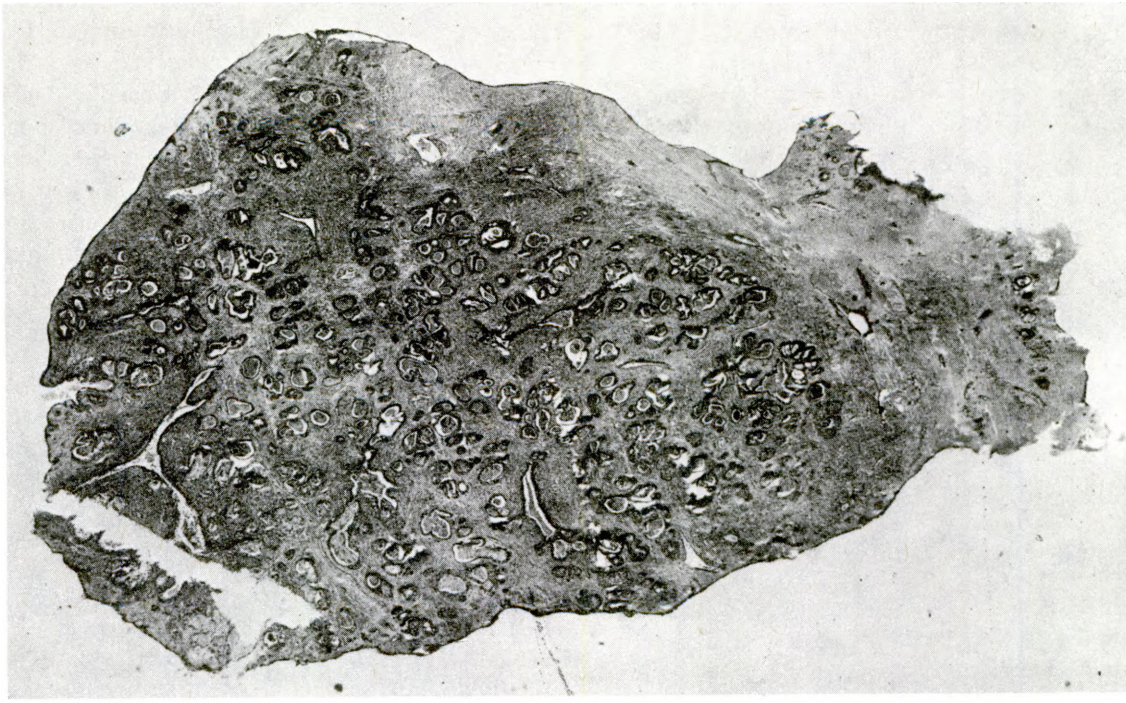

FIGURE 1. Organ culture of human benign nodule 14 days culture (X20)

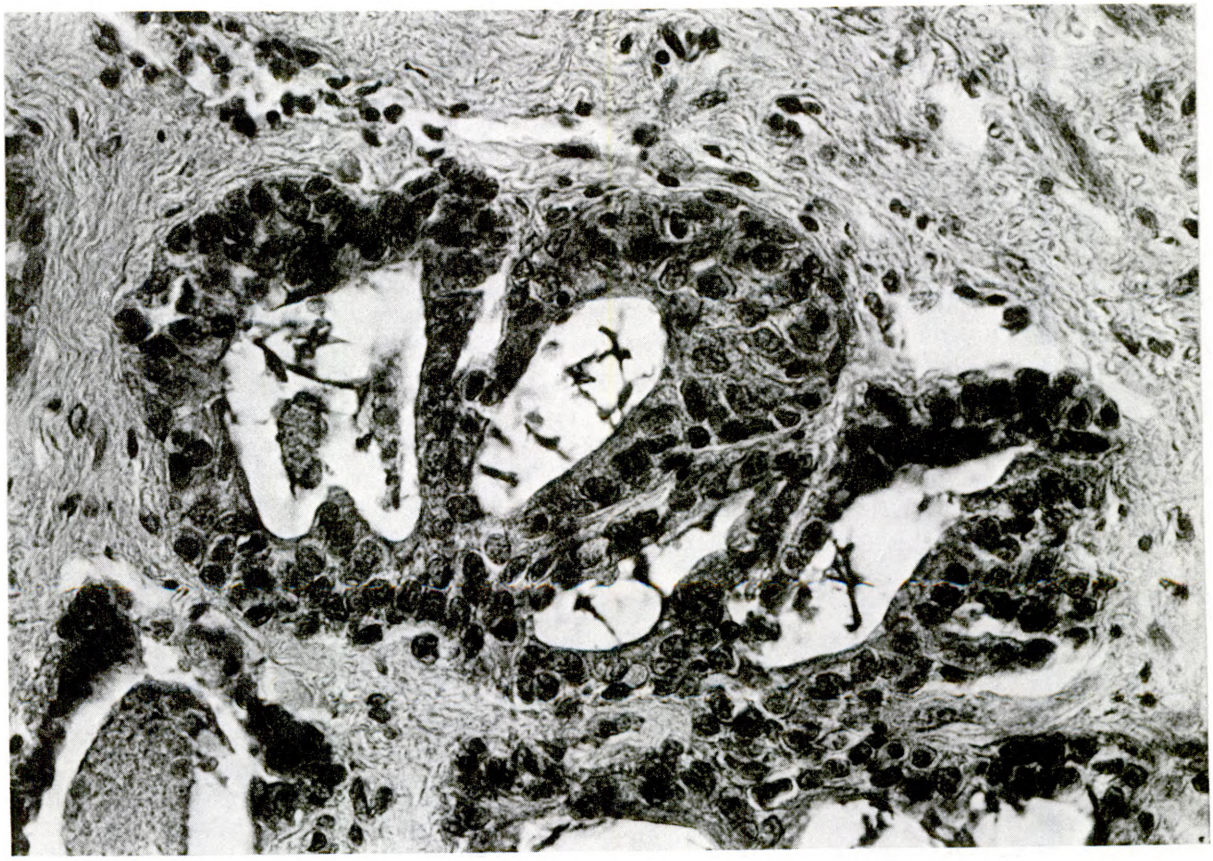

FIGURE 2. An area from the same culture showing mucinous metaplasia (X500)

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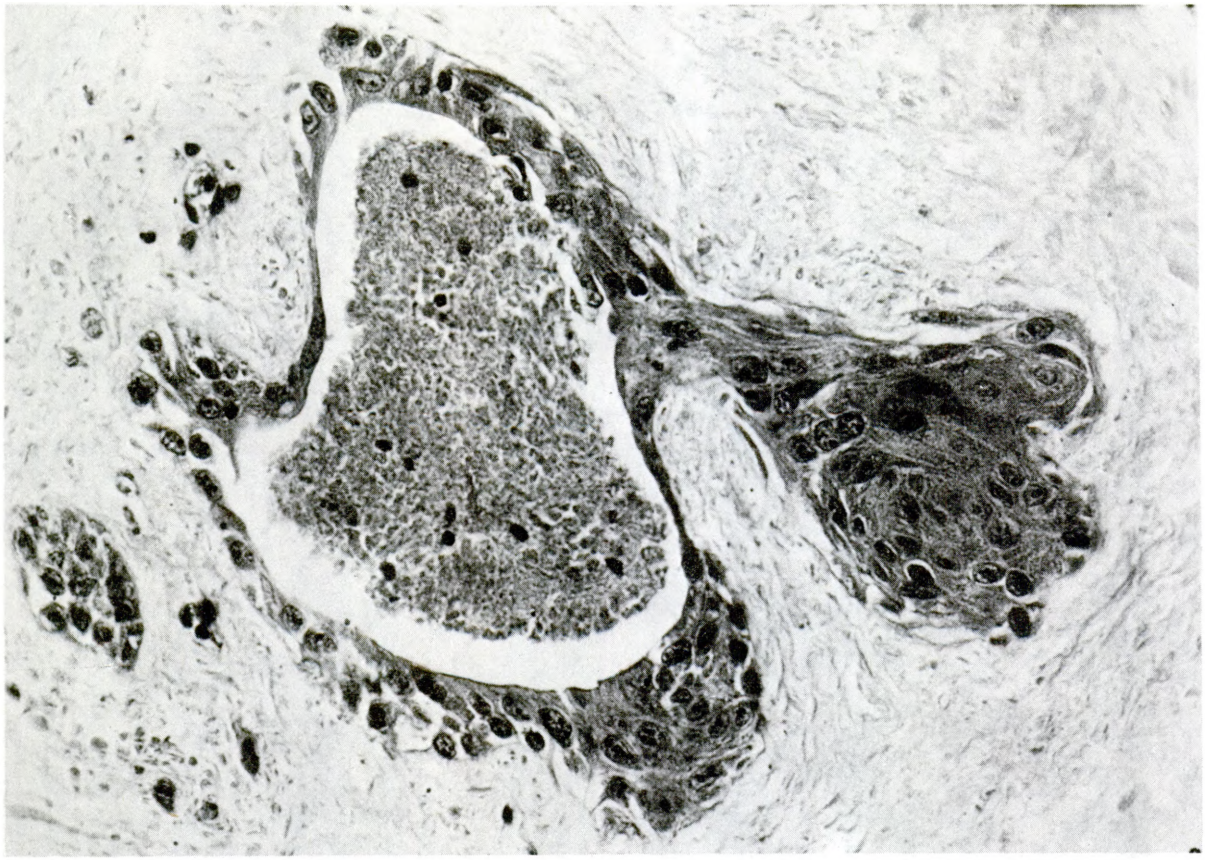

FIGURE 3. Early squamous metaplasia in a similar culture (X500)

tigate the influence of the stroma on epithelial growth. We have discussed this elsewhere in detail (5), but the most significant point is that separated epithelium although morphologically normal (Fig. 5) even under the electron microscope, fails to grow and dies within a few days.

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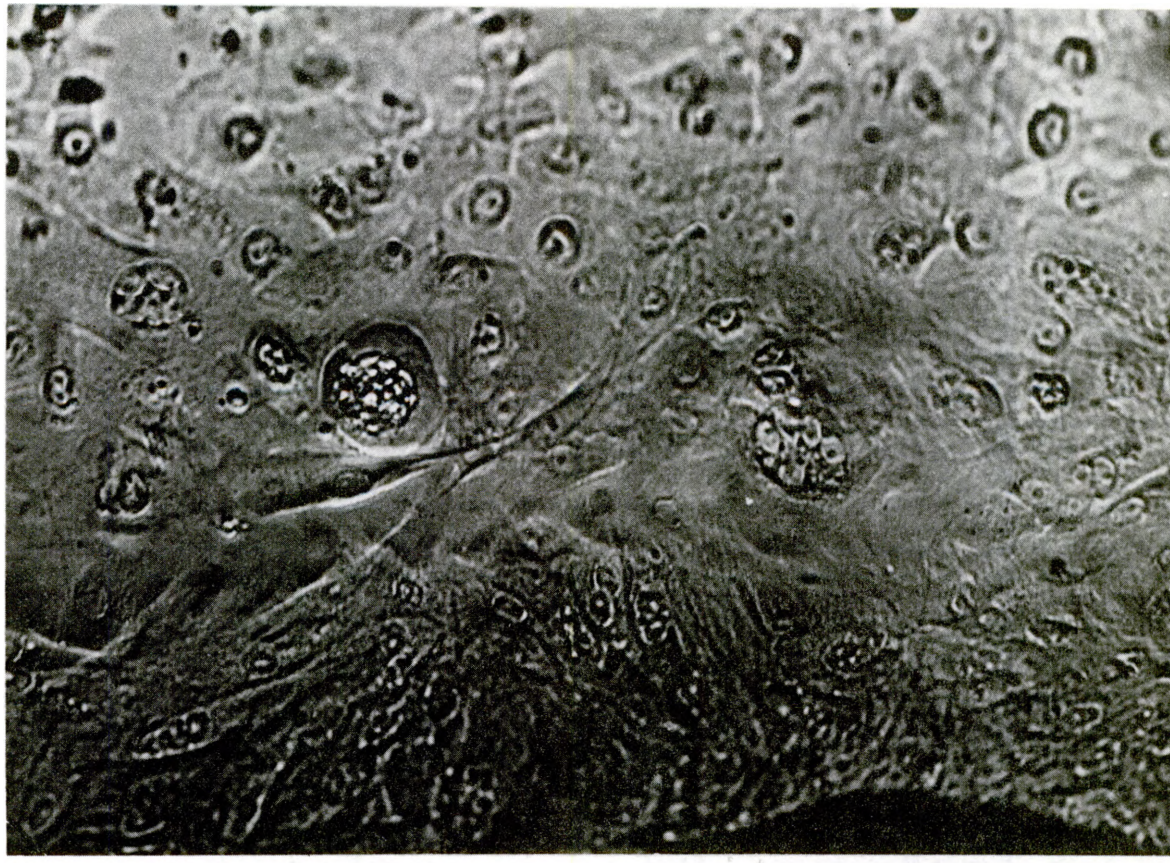

FIGURE 4. Giant cells in epithelial outgrowth for benign nodule.

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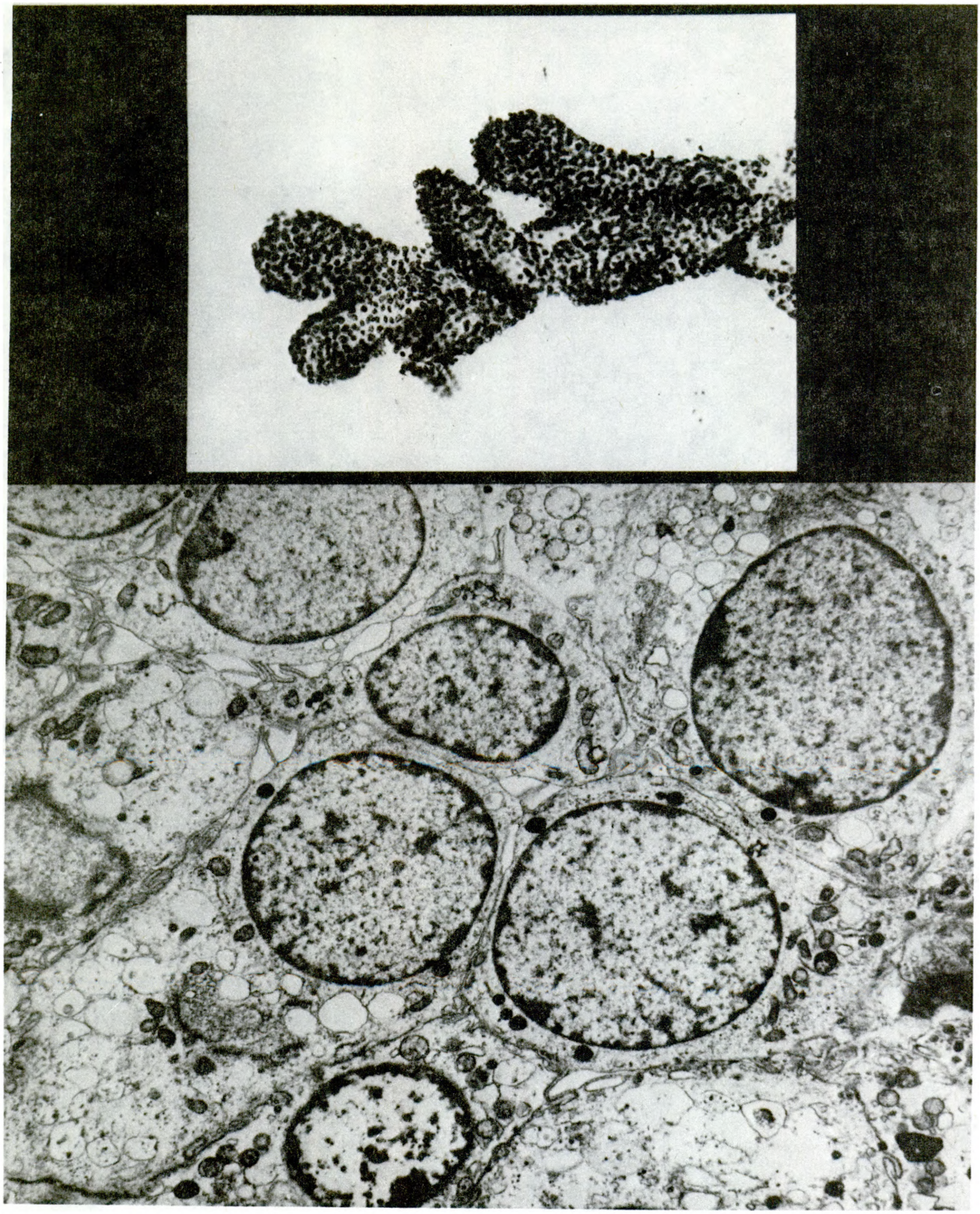

FIGURE 5. Separated epithelium A. X 200 B. X 10,000

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EXPERIMENTAL APPROACHES to B P H: ORGAN PERFUSION

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In vitro and in vivo vascular profusion has been utilized by many investigators to study the physiological, biochemical and functional aspects of organ systems. Perfusion models are useful in that many of the variables present in in vivo preparations can be controlled more accurately. However, new and frequently unrecognized variables are also introduced with the use of new experimental systems. In 1920, Belt stated in the discussion of the perfusion experiments he performed: "We wish to keep constantly in the readers mind that physiological perfusion of any organ is a matter of extreme difficulty, and often great confusion is introduced by such methods which are intended to simplify the study of organ function". (1)

Previous studies of prostatic physiology and function have generally been performed in whole animal preparations. More recently in vitro tissue or organ culture preparations have been utilized to control many of the significant endogenous factors present in in vivo experiments. However, controversy certainly exists as to the similarity of these preparations to the intact functioning organ, and their advantages and disadvantages will surely be emphasized by other discussants. Use of a controlled environment with an intact functioning organ in perfusion studies represents a significant advantage; nevertheless, it is important to recognize the disadvantages of the system as well. Removal of the prostate from an animal or dissection and isolation of its vasculature interrupts the autonomic nerves supplying the gland and destroys any influence they may have on prostatic function. The experimental observations may be altered by effects of extended perfusion on glandular function in a manner not readily apparent.

In this review an attempt will be made to summarize the various techniques of prostatic perfusion that have been used and to discuss the present state of the art; however prior to initiating a discussion of the different models, a brief review of the basic components necessary to any perfusion system seems in order. Obviously the animal model utilized must be adaptable for organ perfusion. Most prostate studies have been performed in the dog because of the ready availability of the animal, the accumulated knowledge of basic canine prostatic function and the ease in which the prostatic circulation can be isolated. Farnsworth however has reported in vivo prostatic perfusion in the rat which will be discussed later in this review. (2)

APPARATUS

The major components of any perfusion apparatus are an organ chamber, a pump to circulate the perfusate, an oxygenator, a cannula or catheter for vascular connections, a reservoir for the perfusate, a filter for the perfusate, a system to regulate and monitor perfusion pressure and flow, and a constant temperature water bath to heat the perfusate.

ORGAN CHAMBER: The organ chamber is required to provide a stable environment for the perfused organ. Generally, the perfusate is warmed just prior to entering the organ by passing through a controlled temperature water bath usually at 37° to 40° C. Many different containers can be used as an organ chamber ranging from a simple disposable plastic structure to a more permanent one with insulated surfaces. Suspension of the chamber in

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the water bath is used in some systems to help maintain a constant internal temperature.

PUMP: Factors important to pump selection are the characteristics (e.g. pulsatile) and rate of flow, the pressure head, and resultant trauma to the perfusate, as well as cost and reliability. Positive displacement rotary or reciprocating pumps, calibrated in such a way that at any given setting output is constant over the physiological 60–160mm Hg range of perfusion pressures, are preferable. (3) This allows easy detection of alterations in vascular resistance by pressure changes since flow rates will always remain constant.

Studies of the mechanical factors involved in trauma to the perfusate are usually measured by the degree of hemolysis that occurs under a particular set of controlled conditions. Erythrocytes have a high tolerance to intermittent positive pressure, midstream turblance and shear stress; wall or surface interaction appears to be the most important factor in initiating cellular damage (3). More blood destruction can be predicted in systems utilizing pumps with valves or occluding or touching surfaces and in systems in which the perfusate is exposed to large surface areas. (4)

The importance of pulsatile or nonpulsatile flow in artificial perfusion has been the subject of much debate. In 1903 Brodie stated that perfusion should be pulsatile for teleological reasons and noted that edema was minimized in organs perfused in a pulsatile manner (5). Some of the undesirable effects attributed to pulseless flow include: increased renin production by the kidney (6), increased systemic peripheral resistance (7), decreased oxygen consumption (8), increased lactic acidosis (9), decreased urine output and sodium excretion (10), and decreased lymphatic return (11). However others have clearly demonstrated the lack of effect of pulseless flow on many of these same parameters (3). Most investigatiors have continued to use a pulsatile pump empirically to attempt to duplicate physiological systems.

OXYGENATOR: Successful maintenance of organ function requires a system capable of adequate oxygen and carbon dioxide exchange. The gas mixture of 95 per-cent oxygen and 5 per-cent carbon dioxide is usually used. Different oxygenators utilize bubble, disc, roller or membrane systems; each has its advantages and disadvantages. Like the pump, the oxygenator has the capability of damaging the perfusate either by the action of a direct blood-gas interface or by the exposure of the blood to a large surface area of synthetic membrane. It is important to emphasize that the oxygenator should be of an appropriate size to handle maximum expected blood flow adequately. If it is too small improper gas exchange with resultant tissue hypoxia will occur; if it is too large excessive blood damage will occur. Construction of special oxygenators to meet the particular requirements of the system is occasionally necessary.

CANNULA: Most perfusion systems require some means of cannulating directly the arterial and venous systems of the organ to be perfused. The ideal tubing should be inert, inexpensive, flexible, impermiable to gases and available in a wide variety of sizes. The pressure-flow relationships of the catheter are important factors in the perfusion system, especially when there is a great disparity between the internal diameter of the cannula and the vessel to which it is attached. Systems utilizing small diameter catheters and high flow velocities increase hemolysis, and systems utilizing thin wall, rigid, non-kinking materials with antithrombogenic surfaces decrease hemolysis and thrombosis. (3).

RESERVOIR FOR THE PERFUSATE: A reservoir is required to help prime the system with perfusate to maintain continuous flow. The volume of perfusate accomodated is dependent upon the amount of fluid added to or removed from the system during the course of an experiment. The position of the inlet and the outlet should be such as to permit sufficient mixing of the perfusate.

FILTER: Filters are required to remove emboli that are present or are formed in the perfusate. They should cause a minimum of trauma and permit full flow of the perfusate.

PRESSURE AND FLOW MONITORING DEVICES: With use of positive displacement pumps, flow can be controlled and kept constant; however, pressure monitoring is required to detect changes in the vascular resistance of the perfused organ. Simple mercury manometers are usually adequate to serve this function, but more sophisticated measuring techniques are available.

WATER BATH: As already discussed, the perfusate is generally passed through a constant temperature bath just prior to its entry into the organ. This helps to maintain the temperature of the perfused organ which can also be monitored if desired.

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PERFUSATE: An ideal perfusion fluid should be chemically defined and have the properties of animal blood. It should have oxygen carrying capacity, contain buffer systems to maintain proper pH, possess osmotic properties to prevent edema and provide nutrients to the perfused organ. Animal blood, utilized by many workers as a perfusate, has the advantage of a great oxygen carrying capacity but has the disadvantage of containing many unknown components that are uncontrollable by the investigator. In studies of prostate physiology pituitary, adrenal and testicular secretions are important factors that must be considered. Use of blood as a perfusate has been associated with protein denaturation (12), erythrocyte and platelet aggregation (13), viscocity changes (14), fat embolism (15), and hemolysis (16), all of which tend to increase peripheral vascular resistance and cause diminished perfusion with resultant cellular damage. Electrolyte solutions with or without the addition of osmotically active agents are frequently used, but their inability to transport sufficient quantities of exygen to the perfused organs is a disadvantage. Either red blood cells or an oxygen-carrying substance must be added to the solution to provide this capability.

The recent discovery of perflourinated liquids and nonionic detergents has shown these substances to be very useful in maintaining a perfused organ in its functional state for a limited period of time. (17,18). Flurocarbons are hydrocarbons that have had all the hydrogen atoms replaced by flourine atoms (19); they may be cyclic or straight chain compounds. Because of the strength of the carbon-flourine bond, flourocarbons are extremely inert, chemically and biologically. They are immiscible with most other liquids and are poor solvents for most substances; however, the solubility of gases, such as hydrogen, oxygen, nitrogen, and carbon dioxide is greater in flourocarbons than in other types of non-polar solvents. The high oxygen solubility has made flourocarbons very useful in "liquid breathing" experiments in which animals survived total submersion in oxygenated organic fluids. (20)

Non-ionic molecular weight detergents have been used in a number of biologic situations including the enhancement of cell growth in tissue cultures (21), stabilization of fat emulsions for intravenous administration (22), prolongation of the safe storage period for blood (23), and in the prevention of fat embolism and hemolysis during extracorporeal circulation (24). Most of the agents are polyoxyethylene or polyoxypropylene derivitives and are available with varying molecular weights and physical properties, depending on the ratio of each moiety. These agents are useful in emulsifying the flourocarbon and as oncotic agents. The other constituents of the perfusate in the flourocarbon—pluronic emulsion consist of electrolytes and buffers to maintain optimum pH and glucose to provide nutrients to the perfused gland. Standard tissue culture media containing buffers, carbohydrates and essential and non-essential amino acids can be substituted in place of an electrolyte solution when preparing a totally synthetic flourocarbon-pluronic perfusate.

EVALUATION OF VIABILITY

Multiple parameters may be utilized to evaluate the adequacy of perfusion in maintaining organ viability. Some, such as organ appearance, are subjective; more objective parameters can and should be utilized in monitoring organ survival. Gland viability must be established in any system before reliable physiological or functional investigations can be undertaken. The criteria generally used are: anatomic studies including studies of blood vessels to determine vascular distribution of the perfusate; evidence of cellular preservation such as light and electron microscopic observations and biochemical studies to determine release of enzymes, lysosomes and vasoactive materials; metabolic parameters such as oxygen and glucose utilization and carbon dioxide and acid production; hemodynamic factors including measurements of vascular resistance, edema formation and blood flow distribution; and secretory and metabolic function.

PERFUSION MODELS: In reviewing various prostatic perfusion models it is helpful to differentiate between in vitro and in vivo perfusion systems. In vivo prostatic perfusion experiments generally employ in situ isolation of the venous and arterial blood supply; usually substances are infused directly into the distal aorta, and venous samples are obtained from the vena cava. Perfusion is dependent on maintenance of the animal's circulation. Another type of in vivo system is one in which the aorta is ligated and cannulated and perfused with blood or a synthetic perfusate which is circulated by means of some type of perfusion apparatus. In these experiments the gland is left in situ and remains under the influence of endogenous nervous factors and probably also humoral factors reaching it via unrecognized small vessels. In vitro perfusion systems utilize organs that have been surgically

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removed from the animal and are devoid of all endogenous influences. The glands are placed in a perfusion apparatus and are maintained by methods that have already been discussed.

Hudson and Scott first perfused the canine prostate in 1950 and used both in vitro and an in vivo systems (25,26). They severed the prostatic urethra and bladder neck, isolated the prostatic circulation by ligation of the arterial and venous branches of the hypogastric vessels, and cannulated the lower portion of the abdominal aorta and vena cava. Prostatic fluid was collected through a urethral cannula. Blood was used as the perfusate in in vivo studies (Fig. 1). In in vitro studies a pusatile mechanical pump and a perfusate of a freshly prepared Krebs-Ringers solution were used. Gland viability was determined by comparing values of oxygen consumption and carbon dioxide production of tissue slices obtained prior to and after perfusion. Patency of the entire vascular bed was demonstrated by perfusing the prostate with a buffered solution containing sodium phenolphthalien phosphate just prior to termination of the experiment. The gland was then sharply divided, and an alkaline solution was poured over the surface. All areas of the gland perfused with sodium phenolphthalein phosphate turned red. Investigations were performed studying acid phosphatase and its possible role in intermediate carbohydrate metabolism of the prostate gland and its secretions.

Behnam and associates used a preparation similar to that described by Hudson (27,28,29). The aorta and vena cava were cannulated with tygon tubing, and the gland was perfused in situ. The perfusate consisted of the animal's blood that was circulated with a sigmamotor pump and oxygenated with a surface oxygenator. Neither temperature of the perfused blood nor pH were regulated. Perfusion pressures were maintained at 80 to 90 millimeters of mercury in the arterial circulation. Radioactive phosphorous (p^{32}) uptake was measured and was found to be greater in the inorganic form and in the oxygenated gland. Subsequent studies utilizing microradioautography demonstrated intra-epithelial deposition of diethylstilbesterol phosphate.

Farnsworth et. al., reported an in vivo perfusion model utilizing the rat and described a technique for

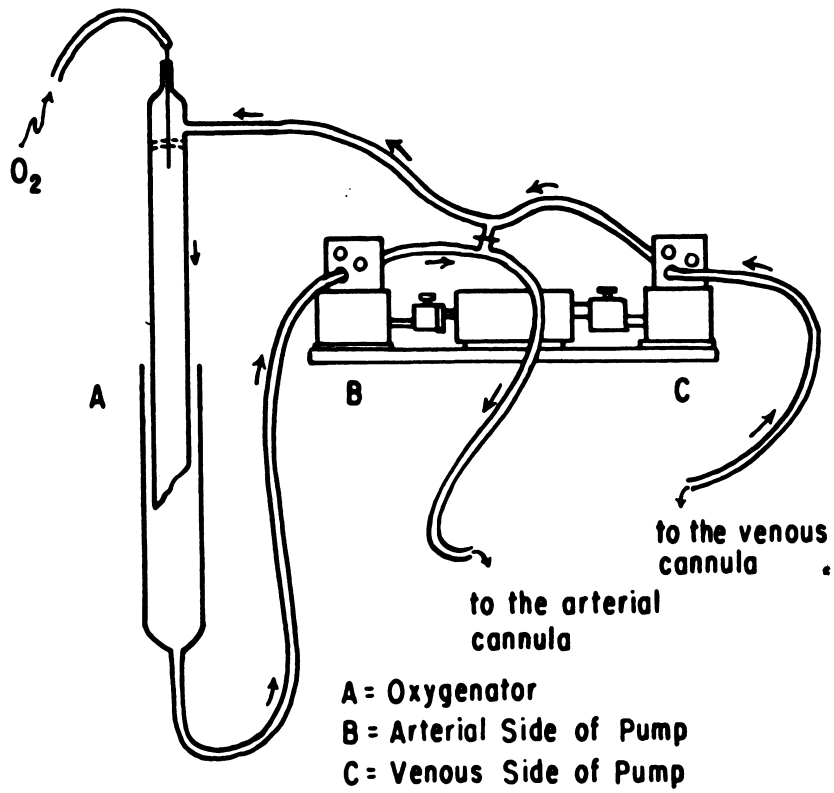

FIGURE 1. Diagramatic representation of apparatus used by Behnam and associates. From J. Urology 84: 6, 1960.

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isolating the arterial and venous system. (2, 30) (Fig. 2A and 2B). The perfusion apparatus was well designed; blood was heated to 37°C, pressures were monitored and maintained at 90–120 mm of mercury, and the perfusate was circulated with a sigmamotor pump and oxygenated with a surface oxygenator. Animal blood was used as the perfusate. Completeness of perfusion was assessed by pumping a saline solution of fast green through the vessels. The entire prostate was stained, and aside from a small region of the bladder wall adjacent to the bladder neck and a small segment of the muscles of the dorsal body wall, no other structures were penetrated by the dye. Criteria of a healthy gland were maintenance of its normal color and the deepening in color of the venous blood. Glands were successfully perfused for an average of 2 to 2½ hours. Investigations were performed to determine the fate of infused testosterone.

In 1970 Morfin and associates described an in vivo model in which the circulation of the dog prostate and bladder was isolated, radioactive androgenic substances were infused into the distal aorta, and the vena cava blood was sampled (31) (Fig. 3). The glands were perfused with the animals' circulating blood. [C^{14}] testosterone was infused into the distal aorta, and assay of prostatic tissue yielded almost exclusively 5α-reduced products, whereas metabolic transformations in the urinary bladder were more limited and primarily oxidative in nature.

Haltmeyer and Eik-Nes described a truly in vitro dog model in which the prostatic arteries were cannulated directly (32). The perfusate was animal blood, which was heated to 37°C, oxygenated, and circulated with a constant rate infusion pump at a rate of 7.8 ml/min. The gland was maintained in an organ chamber, and venous blood was collected in 30 minute samples. Prostatic secretions were col-

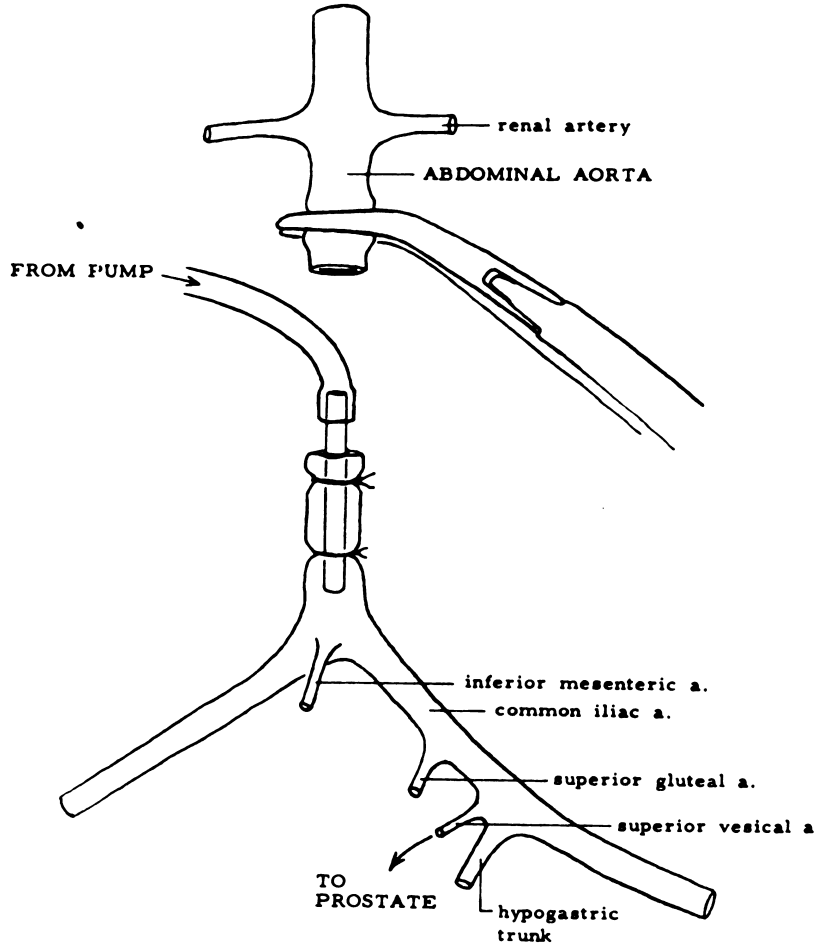

FIGURE 2. A. Diagram of arterial blood supply of rat pelvis cannulated for perfusion. From Endocrinology 73: 498, 1963. B. Schematic diagram of perfusion apparatus of Farnsworth and associates. From Endocrinology 73: 498, 1963.

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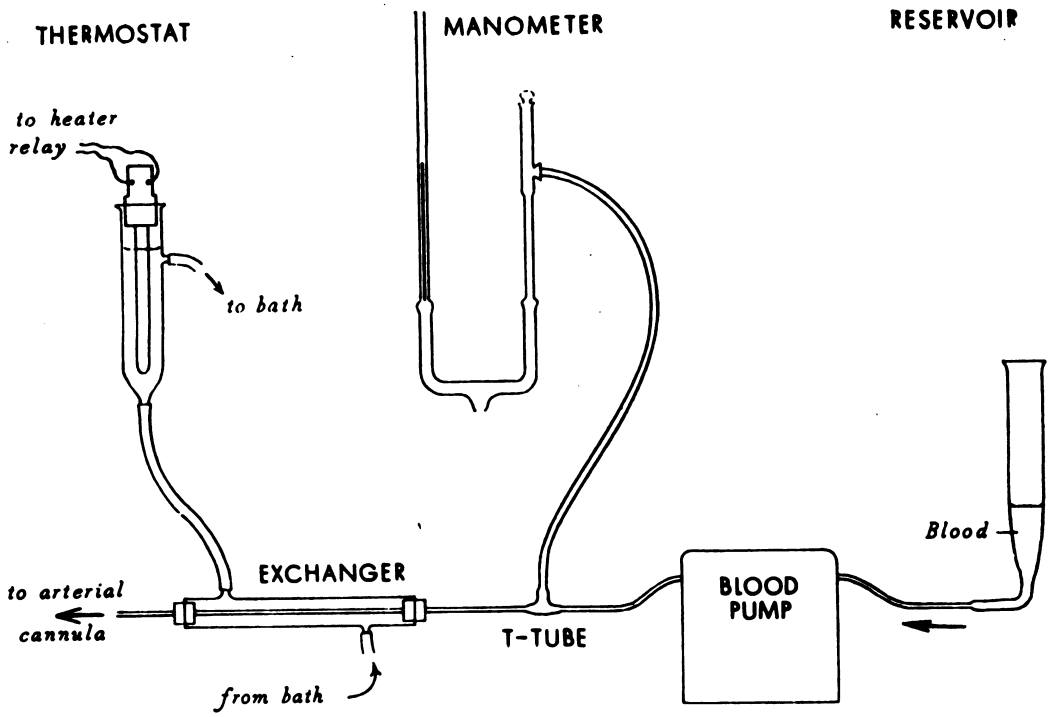

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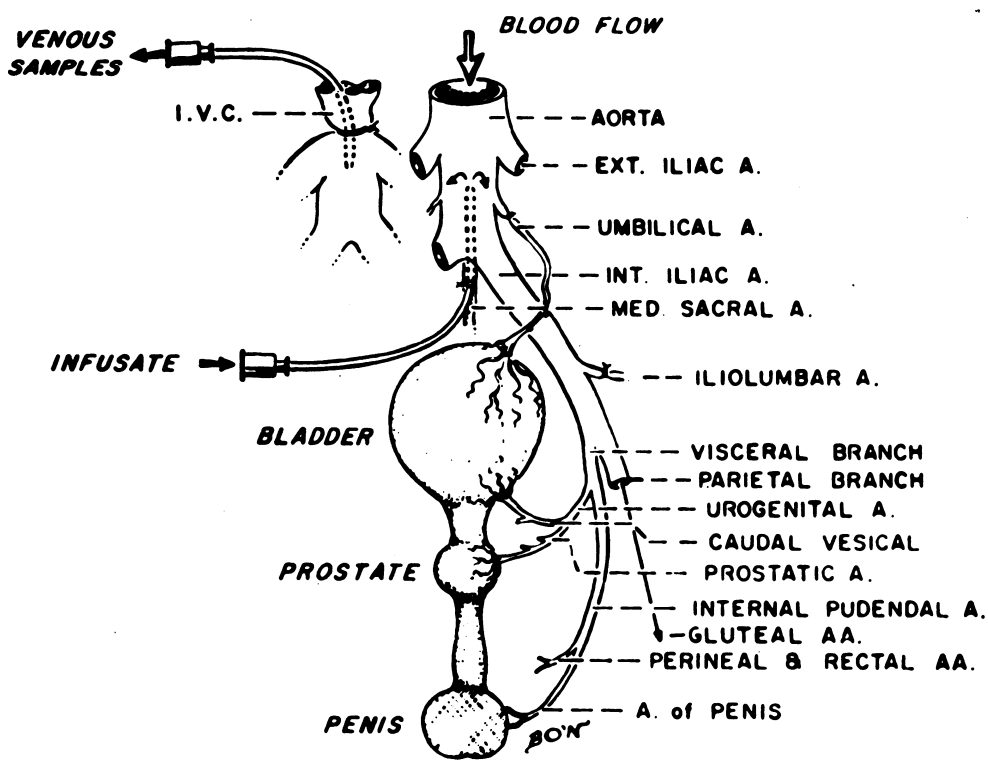

FIGURE 3. Schematic representation of infusion procedure of Morfin and associates. From Endocrinology 87: 394, 1970.

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lected via a cannula in the prostatic ducts. Studies with radioactive testosterone demonstrated that the gland secretes 5α-dihydrotestosterone into the venous blood. No effect of prolactin on the secretion pattern of testosterone or 5α-dihydrotestosterone by the perfused prostate could be demonstrated.

The final perfusion model to be discussed was one developed in our laboratories (33,34). The gland was removed from the dog with an intact vascular pedicle originating at the distal aorta. The apparatus used for maintaining the gland consisted basically of a surface oxygenator, a persistaltic perfusion pump, an organ chamber and a water bath that was maintained as 40°C. (Fig. 4) The perfusate consisted of a flourocarbon-pluronic-electrolyte emulsion. Tissue culture media 199 was substituted for the electrolyte solution to carry out studies measuring radioactive leucine incorporation by the perfused gland. Glands were successfully perfused for a period of six hours. Adequacy of perfusion and gland viability were demonstrated by anatomic studies of the vessels utilizing a self-hardening injectable plastic (Fig. 5), histologic studies to determine cellular preservation (Fig. 6), and functional studies measuring the secretory response of the gland to pilocarpire stimulation and the ability to incorporate radioactive leucine into protein. (Table I) Autoradiography was performed utilizing tritiated testosterone; both estradiol and prolactin were demonstrated to increase the incorporation of the androgen into the prostatic cells. One problem encountered with the system concerned the possi-

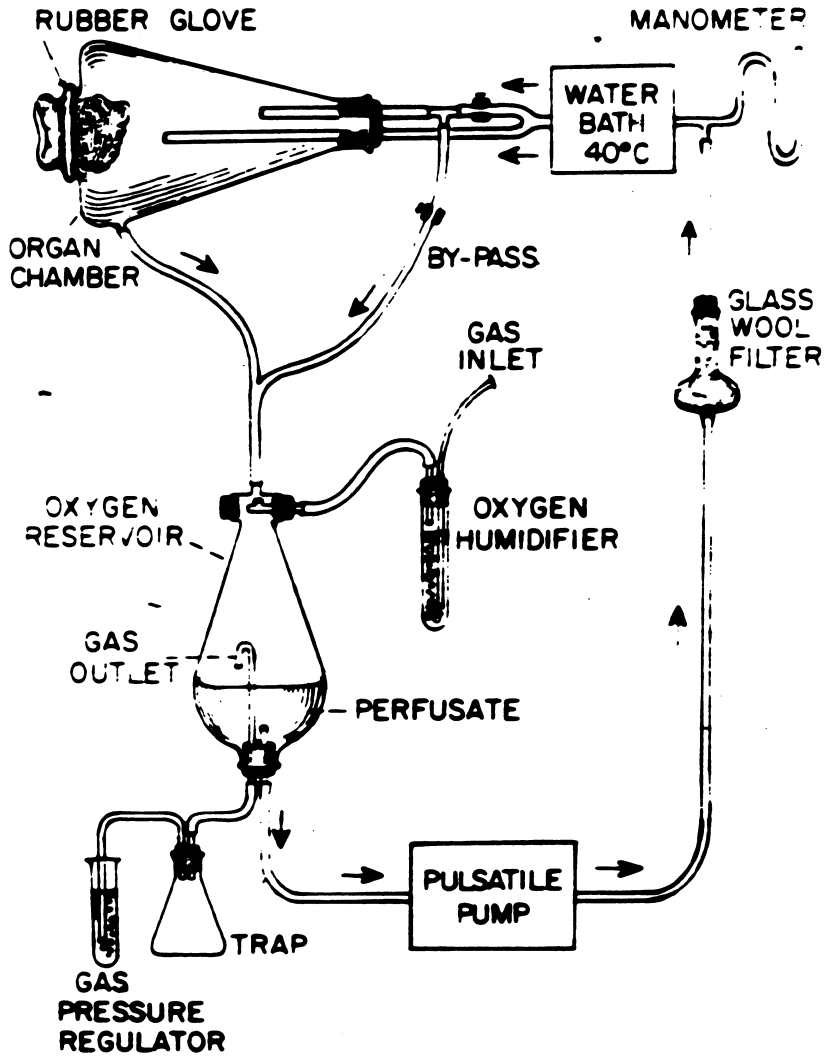

FIGURE 4. Diagram of perfusion apparatus of Resnick and associates. From Investigative Urology 13: 52, 1975.

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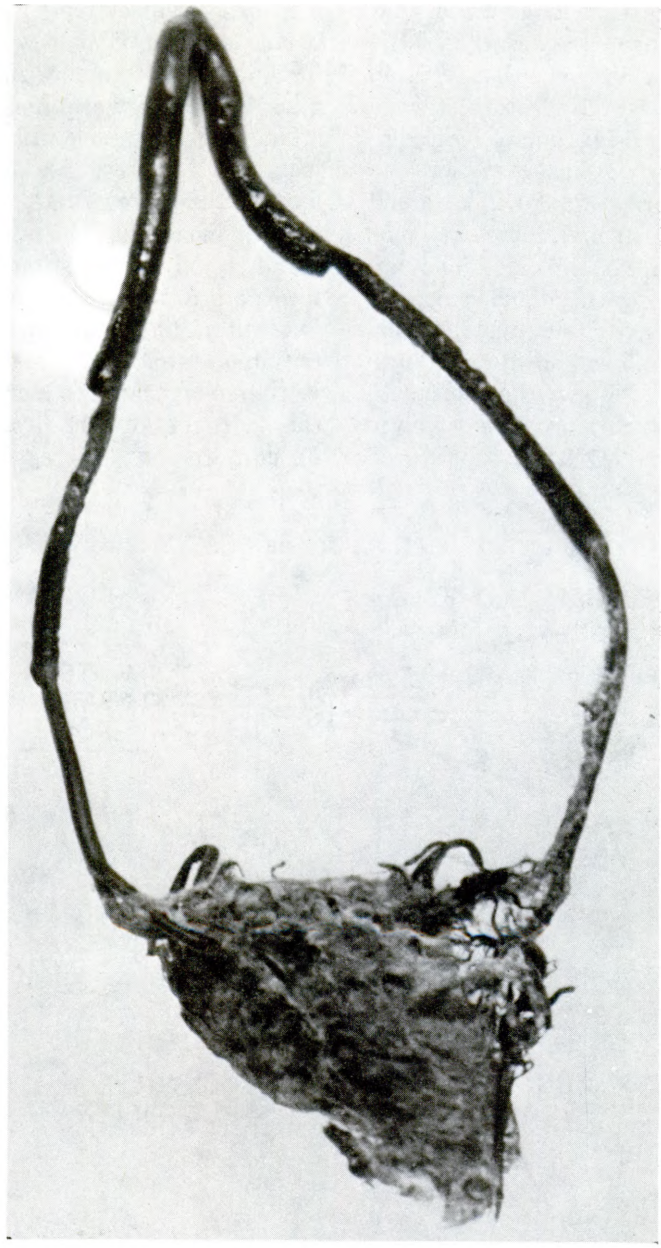

FIGURE 5. Arterial cast demonstrating entire pedicle with uniform perfusion of prostatic parenchmya and lobes of the prostate. From Investigative Urology 13: 52, 1975.

TABLE I

	Volume Prostatic Fluid After 2 Hours Perfusion (ml).	
	Without Pilocarpine	With 10 mg. Pilocarpine
Perfusate with Fluorocarbon	<1	15
Perfusate with Fluorocarbon	<1	6
Perfusate without Fluorocarbon	<1	<1
Perfusate without Fluorocarbon	<1	<1

ble destruction of androgen receptor sites with prolonged perfusion with resultant lack of retention of testosterone within the cells. Further studies are required to clarify this aspect of the model.

The perfusion model offers much potential for further studies of prostatic function and metabolism. Murphy has performed some initial perfusion studies of primate prostates, and interesting data should be forthcoming (35). Scott has recently begun canine perfusion studies and is presently

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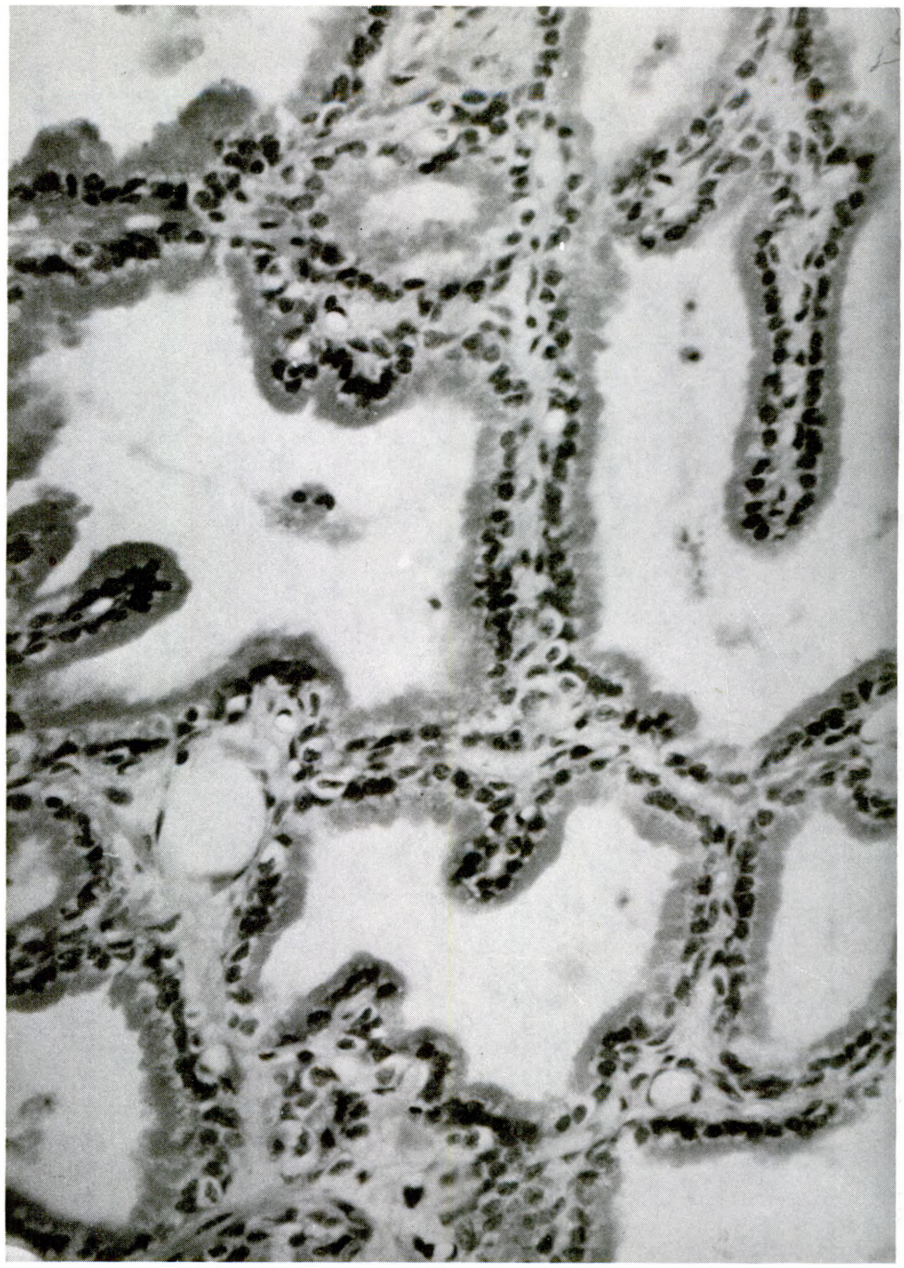

FIGURE 6. Biopsies of prostate: A. at time of surgery; B. after two hours of perfusion; C. after four hours of perfusion; D. after six hours of perfusion.

comparing the quality of the prostatic fluid obtained from the perfused gland with that from the chronic fistula dog and hopes to use the model to study specific pharmacologic agents that may affect prostatic growth (36). If human prostate glands could be obtained from cadavers and surgically removed with an intact vascular pedicle, in vitro perfusion should be possible and would likely be a valuable technique in studying human prostatic function.

Perfusion models have generally been utilized to investigate androgen related metabolism in the prostate. However, these models can be adapted readily towards other areas of interest such as antibiotic uptake or the effect of autonomic stimulating or blocking drugs on prostatic function and metabolism. Perfusion preparations, both in vivo and in vitro, have been used successfully in many different organ systems, and increased efforts in this

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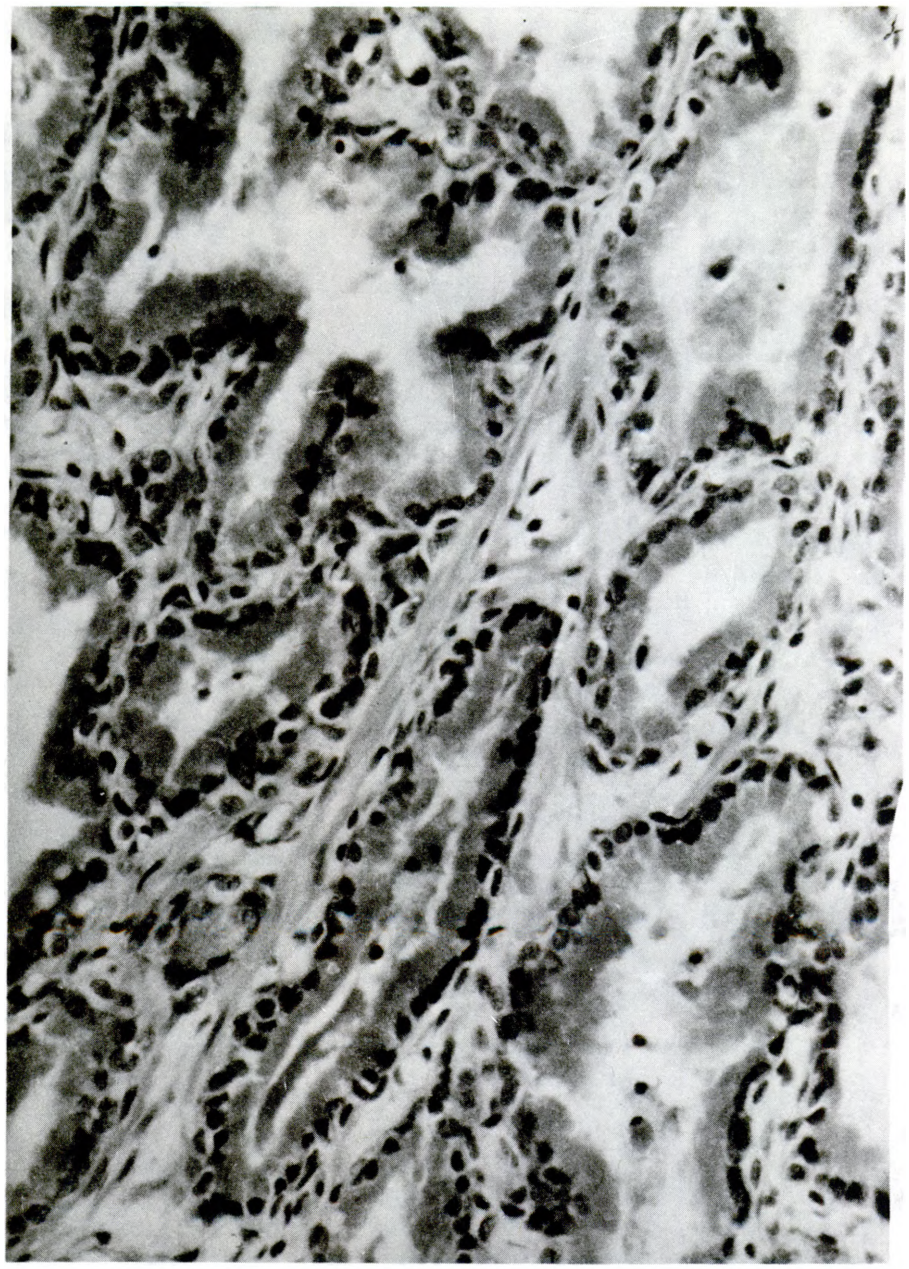

FIGURE 6B—Continued

area will likely establish perfusion preparations as reliable models for studying prostatic metabolism and function.

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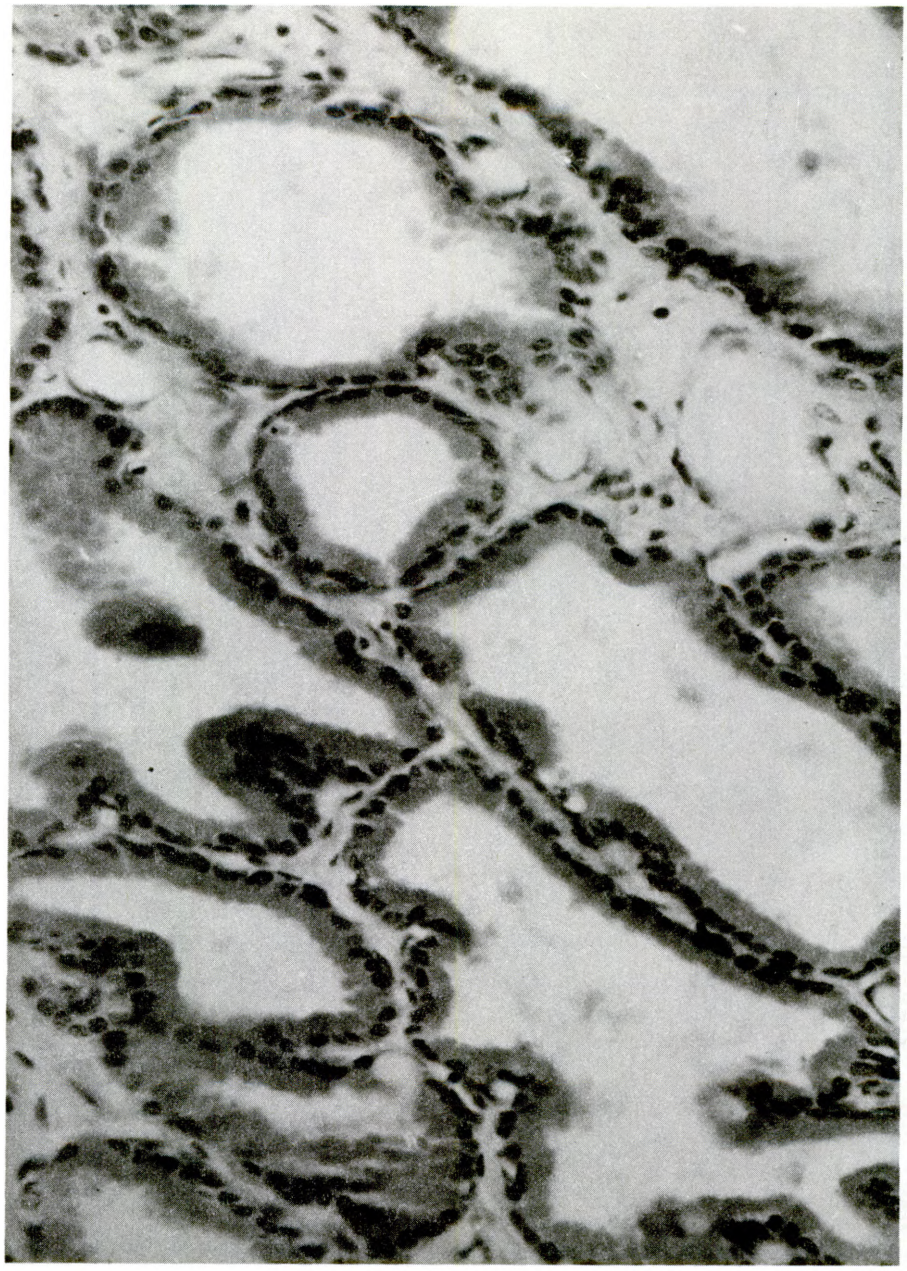

FIGURE 6C—Continued

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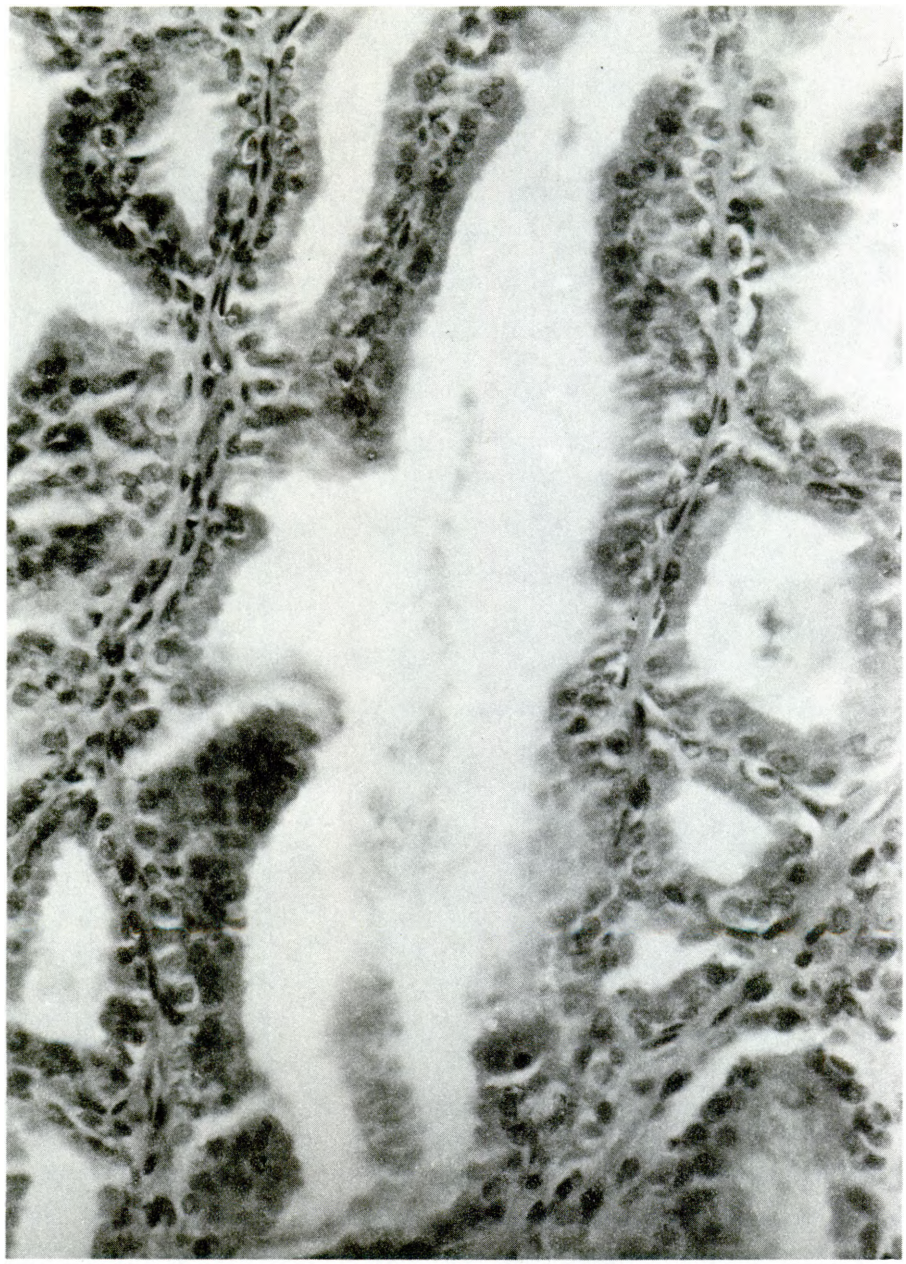

FIGURE 6D—Continued

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DISCUSSION

J.A. THOMAS: Do you advocate the use of fluorocarbons in the oxygenating system?

M. I. RESNICK: We used it in our system as an oxygenator and found it effective in that the entire perfusate was synthetic so that all components of the perfusate were known. Dr. Ewing is performing similar studies using washed red cells as the oxygenator.

THOMAS: Certain fluronated hydrocarbons have an avidity for the prostate. For example, flutamide and a whole series of organochloride pesticides have a significant effect on the prostate, and I wonder if some of the polymers might break down, form smaller hydrocarbons, and interfere with your interpretations.

RESNICK: As I mentioned, with prolonged perfusion the glands failed to retain testosterone, and this may be one of the reasons. We had thought that possibly the receptors were destroyed by the system, and fluocarbons could be one of the agents doing this.

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EXPERIMENTAL APPROACHES TO BPH: IMPLANTS OF BPH IN IMMUNOSUPPRESSED ANIMALS

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Introduction

The heterotransplantation of human tissues into laboratory animals is feasible only when normal immunological rejection is circumvented. Implantation of neoplasms into specialized "immunologically privileged" sites such as the anterior chamber of the eye (1) or the hamster cheek-pouch (2) permitted occasional technical successes. However, at most a transient growth was obtained from very aggressive tumors, and slowly growing implants of normal or hyperplastic prostate tissue were not reported to succeed.

In 1970 Senge reported some successful histological recovery of intramuscular implants of human BPH placed into newborn rats treated with antilymphocyte serum (3). Survival was limited to about 3 weeks, and marked imflammatory reactions were observed in spite of immunosuppression.

More recently, it has been reported that the hairless mutant mice developed by Flanagan (4), called "nude" mice, lacked cell-mediated immunity and could tolerate heterografts of some human tumors (5) and even normal human skin (6) for prolonged periods without inflammatory cell reactions. This report describes some recent and preliminary results from our own laboratory and from others (7) which suggest that it is indeed possible and relatively easy to establish successful, long-term, vascularized implants of BPH in the nude mouse.

Methods

Breeding of nude mice requires special care, but the strain has been easily maintained by several laboratories. We keep the breeding colony in a special room at 80°F in plastic cages covered with air-filtering covers. The mouse food pellets, bedding, and water used are all first autoclaved. Sterile gloves are used when handling the mice and changing between cages. Homozygous nude males (N/N) are mated with heterozygous furred females (N/n), and the weanlings are separated at 4 weeks. The homozygous nudes are clearly distinguishable due to their lack of hair as early as 1 week of age.

Surgery on the nude mice is performed with only token precautions—wiping the instruments and the skin with 70% alcohol. Operated mice have *not* developed wound infection or abscesses with any unusual frequency, suggesting that the precautions in the breeding colony may be superfluous. We have kept caged mice at normal room temperature (70°F) for many days with no precautions other than wrapping the cage in a cloth to reduce the loss of radiated body heat from the hairless animals.

Fragments of benign prostatic tissue are cut sharply with a scalpel or iridectomy scissors to about 1 mm^3 size or to small thin slices not over 1 mm thick in order to avoid central ischemic damage in the implant during the several days before it is re-vascularized by the new host. The tissue is kept

Work supported by Grant PHS CA 15441 and by the Thornburg Medical Research Foundation

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in cold tissue culture medium during the processing and trimming, with technique similar to that used for experimental endocrine tissue transplantations (8). Tissue fragments were placed subcutaneously in the flank.

Testosterone, when administered, was in the form of depot injections of 5 mg of testosterone cypionate, injected at the time of tissue transplantation and monthly thereafter. Male recipients with or without testosterone and females without testosterone were used.

Results

Viable implants of BPH have been recovered in all of the 8 cases of pure BPH processed for transplantation, with histological examination of surgically retrieved implants from 2 weeks to 4 months after initial implantation. Fragments become rounded and protuberant under the overlying skin in 2 to 3 weeks. There is no further progression in size evident after that—i.e. no growth to "tumor" nodules as judged by comparison of serial gross photographs of the animals.

The gross appearance of the implants of BPH under the skin demonstrated good vascularization of the human tissue by mouse vessels, especially well-seen under the dissecting microscope. The 1 mm chips of tissue were larger, 2 to 3 mm in size, due to accumulation of enveloping host tissue. Agglomeration of multiple chips implanted at the same site was commonly found (Figure 1a and 1b).

The microscopic appearance of the BPH retrieved from nude mice displayed a full spectrum of benign glands from those with tall columnar secretory epithelium (Figure 2a and 2b) staining for acid phosphatase (Figure 2c) to many which had clearly undergone squamous metaplasia (Figure 3). There were some areas in which glands displayed an atypia and disorganization which might readily be interpreted as carcinoma (Figure 4) were it not for the fact that the donor tissue was "pure" BPH on mirror sections of the tissue used.

The simplest interpretation of the histological changes observed is probably one of varying effects from the transient ischemia prior to vascularization and perhaps from the continued limitations imposed by the scar tissue reaction. The atypia that suggested carcinoma, for instance, is similar to that pointed out by Franks as occasionally seen in BPH adjacent to an area of prostatic infarction (9).

Viable nodules of tissue appeared also in 6 of 23 cases of implantation of carcinoma of the prostate in nude mice using the same techniques, with or without added testosterone therapy. In each case the nodule appeared with a lag period of 3 to 6 weeks and again failed to grow progressively. Histological examination showed nothing but glands with the appearance of BPH, with extensive stroma between them, but no clear-cut surviving carcinoma tissue (Figure 5a and 5b). Review of the original histology of the carcinoma in these 6 cases indicated that they all had some surviving islands of benign glands infiltrated and surrounded with the tumor (Figure 5c). Significantly, none of 8 cases of carcinoma of the prostate metastatic to lymph node tissue showed any surviving prostatic tissue when the lymph node's tumor was implanted into nude mice.

There was no obvious histological difference in tissue removed from normal males or females or testosterone-treated males. At the intervals studied, the implants removed from females did lack any obvious tall-secretory epithelium, such as could be seen in some fragments removed from males.

Discussion

These preliminary observations establish the feasibility of maintaining fragments of human BPH as heterografts for prolonged periods of at least 3 months. The absence of an immune inflammatory reaction and the excellent vascularization of the implants imply that the grafts will be maintained for the life of the nude mouse. Although not yet done, serial transplantation into other nude mice is clearly feasible and should make it theoretically possible to maintain such BPH fragments for years for various study purposes.

The metaplastic changes observed in many of the glands, particularly the squamous metaplasia consistently observed and seemingly unrelated to the animal's hormonal status, are a disappointing feature of our findings. Histological changes of such degree bring into question the relevance of further changes that might be effected with drugs or hormonal manipulations. Yet the intact morphological preservation of some of the glands, including abundant secretion of acid phosphatase (Figure 2), suggests that the new environment in the nude mouse can maintain BPH in a useful state of preservation. Perhaps details of tissue handling and sites of implantation can be altered to improve early oxygenation and ambient temperature (e.g. transplants to a site deeper than the subcutaneous area).

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FIGURE 1a. Surgical exposure of subcutaneous space of the back of a nude mouse bearing implants of 3 months duration. Skin flaps have been peeled back from the midline to reveal the two lobulated masses attached to the panniculus and criss-crossed with blood vessels. In this example, a 67 year old man's prostatic cancer chips were implanted on the left side and BPH chips ("nl") on the right side. The chips have aggregated to form lobulated masses.

FIGURE 1b. Low power histologic view of an implant of BPH recovered at 5 weeks in a subcutaneous pocket similar to those shown in Figure 1a. From patient R. T. (cf. figs. 2 and 3 also.)

Okada, Schroeder, et al (7) have noted similar results with heterotransplantation of BPH into nude mice. They are also unable to correlate their histology with hormonal manipulation. They have studied in more depth the mitotic activity of the BPH heterotransplants and found very meager (0.1%) labelling of cells with thymidine (10).

Whether the techniques described here can offer any advantage over organ culture (11) remains to be seen. It is likely that the prolonged observation

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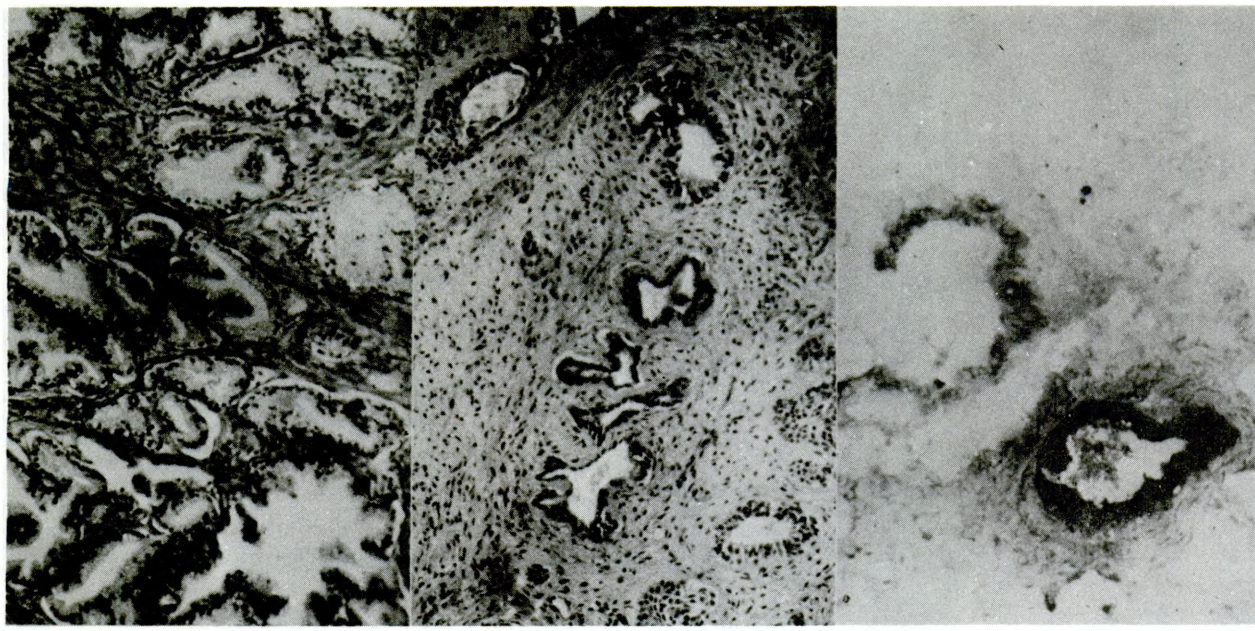

FIGURE 2. Histology of BPH implants in nude mice.

a. Original appearance of BPH tissue implanted—H and E "mirror" section of surgical specimen on patient R. T. H and E, 125X.

b. BPH glands with columnar epithelium surrounded by dense stroma in nodule recovered after 5 weeks in a testosterone-treated male nude mouse. Patient R. T. (see fig. 1b) H and E, 125X.

c. Acid phosphatase stain of tall columnar epithelium in implanted glands similar to those in Figure 2b—200 X.

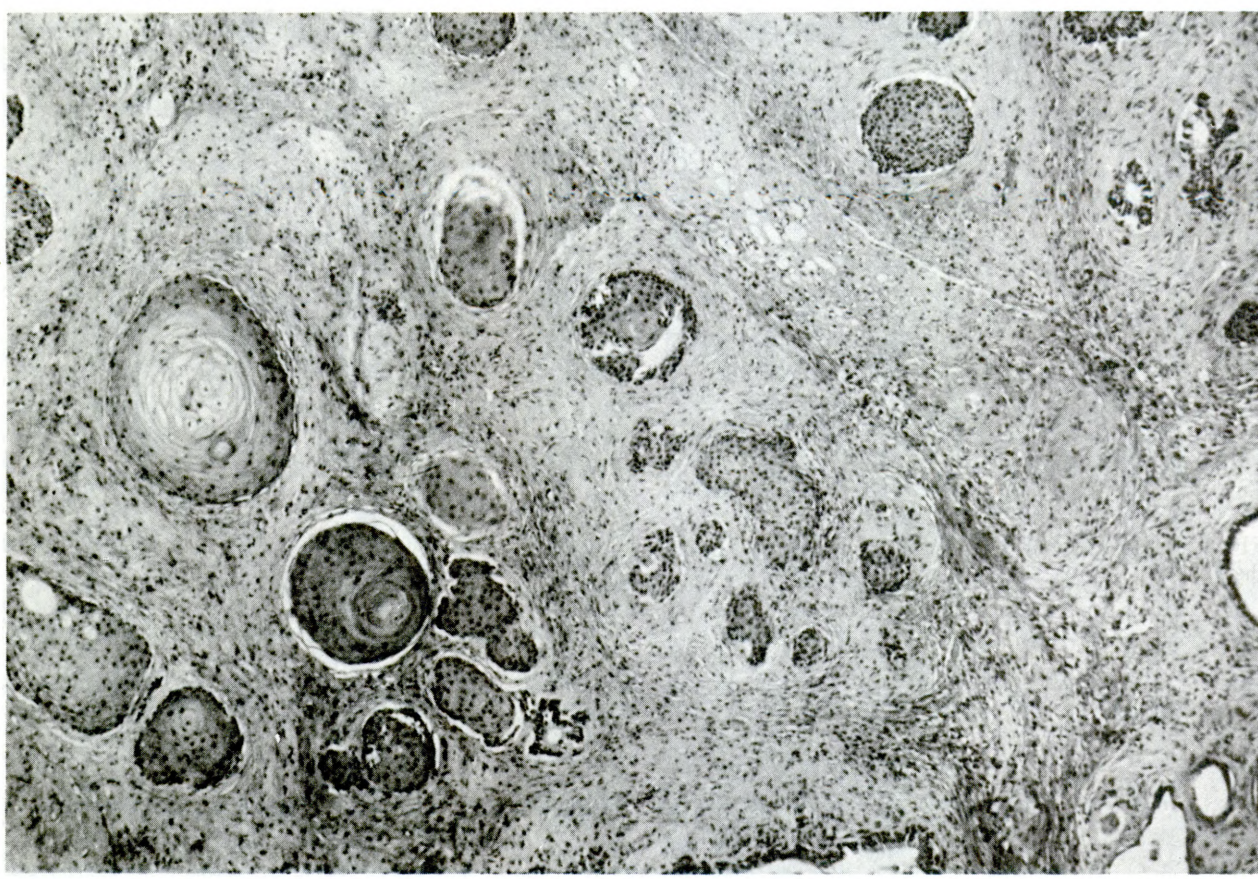

FIGURE 3. Squamous metaplasia of BPH glands shown in a closeup of the section shown in Figure 1b. Note spectrum of histology, from Keratin pearl formation in left center to secretory epithelium in bottom center and right. H and E, 100X.

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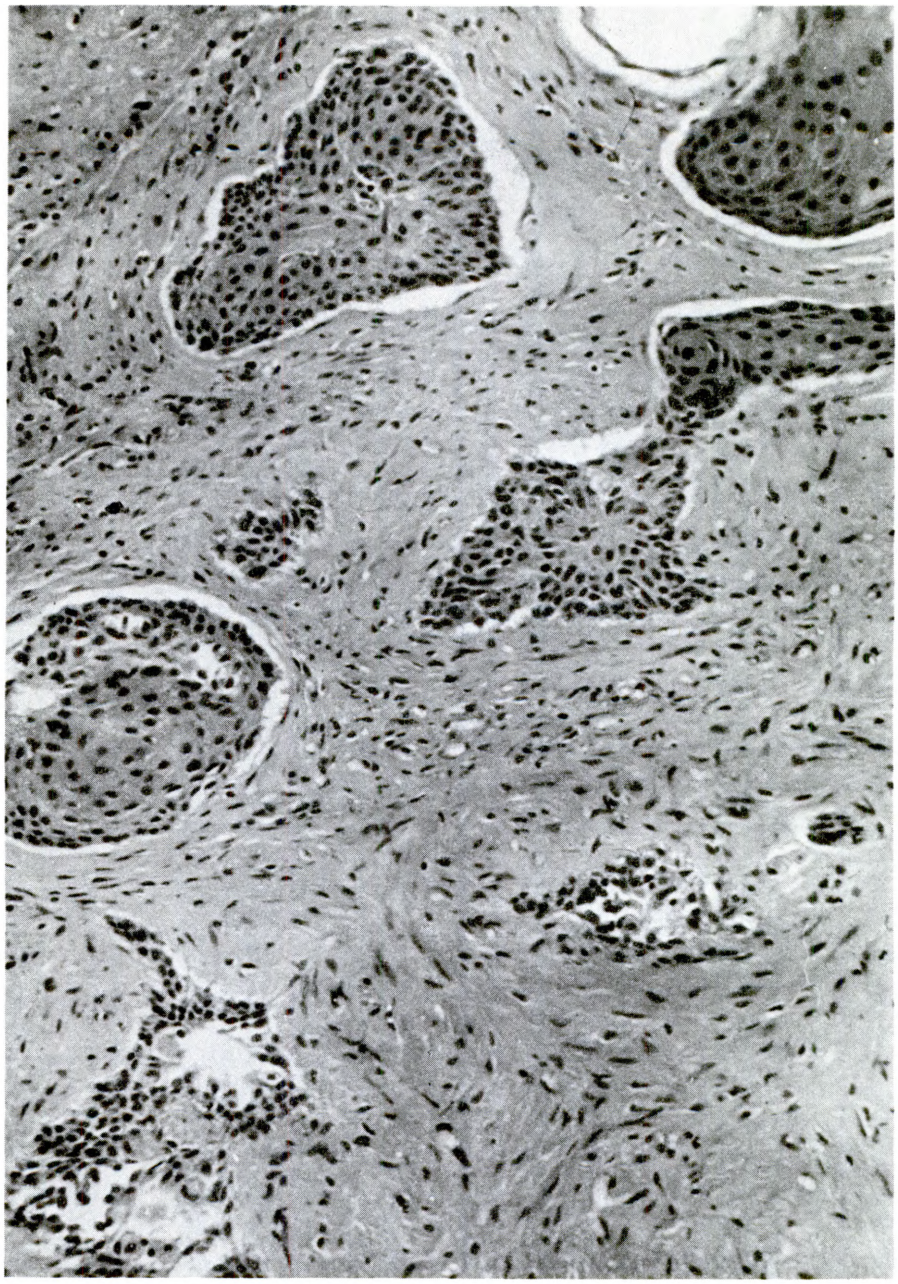

FIGURE 4. Cellular atypia and epithelial disorganization suggestive of malignancy in benign BPH glands, shown in a closeup view of the section shown in Figure 1b, H and E, 200X.

period made possible here will be advantageous in the study of this extremely slow-growing and as yet poorly understood human "tumor".

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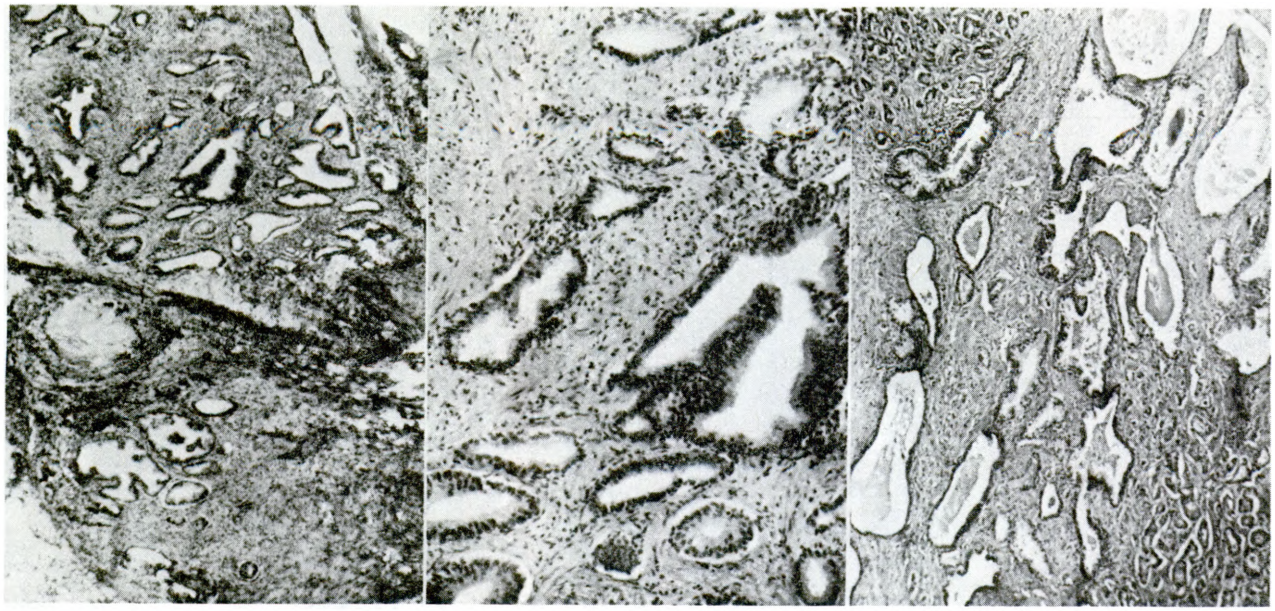

FIGURE 5. Preferential survival of BPH glands in transplants of carinoma of the prostate.

a. Low power histological appearance of nodule removed from left flank of nude mouse (shown in Figure 1a) 3 months after implantation of fragments of primary carcinoma of the prostate. H and E, 8X.

b. Detail of benign-appearing glands in same section shown in (a) H and E, 200X.

c. Section of original histology of tumor implanted into nude mice. The obvious carcinoma in upper left and lower right corners is infiltrating among the benign-appearing glands. It appears that the BPH tissue but not the adenocarcinoma has survived transplantation into the testosterone-treated mouse. H and E, 125X.

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DISCUSSION

F. H. SCHROEDER: We've been doing similar work in our laboratory. Six prostatic adenomas were transplanted into 26 nude mice, and the take rate was 83% for tumors and 77% for mice. The definition of take is recovery of prostatic tissue from the animal. Typically, we find metaplasia on one hand and very well maintained columnar epithelium on the other. We castrate all animals, male or female, and administer 1 μg testosterone propionate per gram body weight per day to half the animals. The other half do not receive any testosterone. The average weight of the prostates used for transplants was 49 grams. We transplant a single piece underneath the muscular fascia. We try to cut the tissue as a flat cube about 4 × 4 × 3 millimeters in size. Recently, we've also transplanted a total of 19 carcinomas into nude mice; 9 of these were transplanted subfascially, and 10 were transplanted subcutaneously. Since we started subfascial transplantations and giving daily doses of testosterone, we have been able to recover prostatic carcinoma tissue on 9 occasions.

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REFLECTIONS ON THE NIH WORKSHOP ON BPH

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The objective of this NIH workshop, as set forth by its organizers, is to stimulate efforts to prevent or control the development of benign prostatic hyperplasia (BPH) and its sequellae on the urinary tract of man.

BPH is a major medical problem for a sizable fraction of the male population; it is the most common benign tumor in the aging male. Men generally develop some degree of prostate enlargement, after they reach the age of 55 to 60 years. By the age of 80, more than 90% will have developed frank BPH with varying degrees of bladder neck obstruction and urinary retention. Despite widespread occurrence, this disease process has not attracted large scale funding or extensive major research efforts, perhaps because BPH is not a "dramatic" disease. It involves older men, who some think have seen their "best days". Moreover not too many of these men, at least in advanced countries, will die because of BPH and its complications. The relatively low mortality is the direct consequence of a relatively safe and effective surgical procedure (prostatectomy) for relief of the bladder neck obstruction produced by BPH (Grayhack and Sadlowski).

Given effective surgical therapy for the clinical problems arising from BPH, why should one devote scarce biomedical resources in an effort to understand the etiology of BPH and develop a medical treatment for its control? Several points need to be made. The first is straightforward economics: it has been estimated that there is a 5–10% probability that a man who is 40 years old will require a prostatectomy if he lives to be 70 or 80; an effective medical treatment would markedly reduce these costs. Second, a significant fraction of elderly men develop obstruction under circumstances in which they are very poor risks for surgery. This group would benefit from medical therapy. Third, although the distribution and availability of surgical expertise and facilities in the US may be adequate (at least in theory) for the treatment of all patients with obstructive BPH, this is not necessarily the case for all regions in other countries in the world. The reported high mortality *due to* BPH in certain countries reported by Rotkin (as differentiated from BPH associated with death due to other causes) is perhaps directly related to this factor. Even in the US, there are many elderly men with BPH who will not undergo surgery for one reason or another. Finally to be considered—and perhaps most important—*a medical treatment of BPH would provide major benefits to the improvement of the quality of life for all aging men.* The male once he reaches a critical age (whether it be 60, 70 or 80) accepts "dribbling", frequent urination at night and other inconveniences which cause him embarrassment and some loss of personal dignity; he resists surgery until it becomes necessary for survival. The psychological complications of BPH suffered by aged men are real. All of us have been "imprinted" during childhood training with the importance of bladder control; for the sizable fraction of our aged male population who have lost control over so many other aspects of their lives, the urinary sequellae of BPH represent a most distressing feature of growing old. Considered in these terms, BPH is a disease process with major costs to mankind and merits significant research funding. A medical treatment for BPH would thus have major medical, economic and psychosocial benefits.

What can be done to develop an effective medical treatment for BPH? The linkage between the tes-

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tis, androgenic steroid hormones and prostate growth has long been established. The well documented evidence that prostate growth in mammals generally is dependent upon androgens has led to widespread acceptance of the idea that BPH might be controlled by a medical procedure if the "right" androgen antagonist could be found. Ever since Huggins used diethylstilbesterol as an anti-androgen in prostatic disorders, there has been a search to find a potent androgen antagonist that inhibits prostate growth without producing deleterious "side effects". Our ideas about the causation of BPH have similarly centered on androgenic hormones. Indeed, the main thrust of the experimental approach to BPH has been based on the assumption that if we "really" understood how androgens and other steroids act to promote prostate growth in aged as well as young animals, we would be in a position to resolve the problem of BPH. Such a view demands detailed study of the mechanisms of steroid metabolism and androgen action in the prostate, particularly as these processes may change with aging. The circulating levels of those hormones that are critically involved in prostate function are likewise of obvious importance in this connection.

In recent years there have been spectacular advances in our general understanding of the successive molecular events involved in the action of steroid hormones; particular emphasis has been directed to the role of steroid hormone receptors which interact with specific steroids to form complexes that serve to initiate action at the geneomic locus. As part of this development the effect of androgens upon the rat ventral prostate has been extensively studied from this point of view; indeed this system has served as the principal model for study for androgen action generally. A metabolite of testosterone, 5α-dihydrotestosterone (5α-DHT) formed within the prostate cell is now widely regarded as the "active" steroid moiety ("intracellular hormone") which interacts with the androgen receptor in prostate to initiate many hormonal responses. The 5α-DHT-receptor complex is believed to be responsible for initiating the androgenic growth in other accessory glands of the male genital tract and additionally in certain other (but not all) androgen target tissues. In androgen-responsive tissues where 5α- and 3α-steroid reductive enzyme systems are absent or low (e.g. muscle) unmetabolized testosterone interacts with androgen receptor to initiate the hormonal response. Thus, testosterone appears to be unique among circulating steroid hormones in that it serves as a "prohormone" for responsive cells in certain target tissues and as "hormone" in others. Concurrently as technics become available to measure the levels of circulating hormones, these have been applied to humans of varying age. Thus it is now possible to describe accurately changes in the pattern of circulating hormones, known or presumed to be involved in prostate growth, which may possibly be involved in the development of BPH.

Given this new information, the workshop brought together experts who have specialized in the study of one aspect or another of clinical BPH and other researchers who have specialized in studying androgen action and/or steroid metabolism to reexamine the problem of BPH from a broad interdisciplinary point of view. The organizers felt that this workshop might provide a unique opportunity to reevaluate the basic assumptions that have guided BPH research. The fundamental issue implicitly posed by this workshop is whether our conventional approach to the causation of BPH, which has emphasized the primary role of the testis and androgens in relation to aging is really adequate. At the conclusion of the meeting, I was asked to appraise critically the research efforts in the BPH field, to define some of the gaps which need to be "filled-in" and perhaps to suggest new ideas and possible new approaches to the BPH problem. I accepted the assignment despite my lack of expertise in the BPH problem because, at my age, I have more than academic interest in BPH.

This essay reflects the *prejudices* and *limitations* of an "outsider on the inside"; it is not a review of papers presented at the workshop. In preparing this essay, I have had the assistance and cooperation of Drs. Jean Wilson, John Grayhack and Guy Williams-Ashman. After I read the manuscripts presented at the workshop, they tried to educate me about the prostate gland, human and otherwise, and a host of related issues. At certain points in our dialogue, when the questions I put appeared to lead in novel directions, one or the other contributed the critical information necessary to develop the "answers" to be suggested in this essay. If this essay has "positive" features suggesting new areas for the experimental investigation of BPH that turn out to be profitable, these should be regarded as the results of the collective dialogue between Wilson, Grayhack, Williams-Ashman and myself. I retain responsibility for all deficiencies present.

I. Some Fundamental Features of BPH

Certain features of BPH described at the workshop which appear to be fundamental in relation to

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etiology, may be illustrated by posing the following questions.

Q–1: *What is the locus of BPH?*

BPH has a distinctive periurethral origin; it very seldom involves the peripheral zones of the prostate gland. This distinction is recognized by urologists who differentiate between the "outer" and "inner" prostate (Scott). The periurethral tissue involved in BPH consists of small epithelial glands that originate as diverticulae of the urethra and that extend radially into the periurethral connective tissue and smooth muscle. The periurethral tissue involved in BPH, though surrounded by the prostate gland proper, may well be distinctive ontogenetically from "true" prostrate tissue as suggested by McNeil.

Q–2: *Is there an invariant cytological feature in BPH?*

The multiple cell types in periurethral tissue are generally subdivided into epithelial and stromal cellular elements. The stromal cells include smooth muscle fibers, myoepithelial cells and the "fibroblast-like" cell types responsible for formation of the collagen and elastin fibers of the interstitial stroma. BPH nodules do not represent hyperplasia of a *single specific cell type.* Instead there are at least five types of BPH nodules as distinguished by the cell types involved (Franks). Thus, nodules may be formed primarily from either stromal elements (fibrovascular nodules), smooth muscle elements (leiomyoma) or as mixed epithelial-stromal elements (fibro-myoadenomatous nodules). If there is a distinctive cytological feature of BPH, it is that in all nodules *the normal architectural arrangement and social relationships between the various cell types present in periurethral tissue are markedly altered.* In effect, the normal growth pattern, which involves coordinated growth and division of neighboring epithelial and stromal cells within the tissue, is disturbed in BPH so that cells grow and divide independently of their neighbors to produce nodules where cells are arranged in a new ordered form but with different architecture. Among exocrine glands, the prostate may not be unique in exhibiting age dependent perturbations in mesenchymal-epithelial relations.

Q–3: *What is the Natural History of BPH?*

Little is known about this important aspect of BPH (Brendler). BPH is seldom seen before age 40; with advancing age the incidence of BPH increases so that almost all prostate glands in men who are 80 exhibit hyperplastic nodules. Some clinicians have the impression that BPH begins early in adult life. If it does, we do not know when. Nor do we know whether the initial changes which lead to BPH involve the epithelial or stromal elements of periurethral tissues. The problem here is that we do not really know what the word "begin" really means in an operational sense. How does one measure "begin" in a living patient? Brendler's educated guess, shared by many urologists, is that a *small* periurethral nodule once formed requires 15–25 years before it is transformed into a bonafide clinical BPH entity. There is likewise a clinical impression that the growth of BPH nodules may be quite variable. In some cases it appears that BPH may be static for years and that this quiescent phase can be followed by resumption of rapid growth; in other cases, there is the impression that BPH nodules actually regress; the factors involved are unknown.

Q–4: *Is BPH an Invariant Feature of Aging in the Male?*

It appears to be, but we really do not know. Nor do we have an explanation why the "symptoms" of BPH are first detected at different ages in different men. Differential loci producing variable "symptoms" is one answer. One may ask, however, whether other factors are involved in the variation observed in BPH detection. Is this the result of differential rates of progression of a small hyperplastic process that starts rather uniformly early in adulthood (say between 20 and 30)? Or is it the result of the initiation of BPH at different ages? Whatever the basis, the variability observed suggests that genetic and/or environmental factors possibly influence the development of BPH. The significance of such factors cannot be evaluated from the limited available epidemiological data on BPH, surveyed by Rotkin.

The importance of the epidemiological approach in relating certain environmental factors to human carcinoma is now well documented. If critically applied to BPH, epidemiological studies might well clarify the role that environmental and/or genetic factors play in the development and the age of onset of obstructive BPH. The available epidemiological data primarily concern "symptoms" and *not* a study of the prostate gland itself. Nevertheless, there are some "hints". In the US, for example, Blacks appear to develop BPH at an earlier age

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than do Caucasians; orientals generally, but more importantly Japanese living in Japan, appear to have a lower incidence of BPH than do Europeans or Americans. If the Japanese data regarding BPH are really "sound", it would be of great interest to compare the incidence of BPH in Japanese living in Japan, Hawaii and the US. Such studies in the past have "pin-pointed" the importance of enviromental (dietary) factors operative in other disease process. A second problem that bears on the issue of the "invariant" nature of BPH concerns isolated regions in the world where humans are said to live to a very old age under very primitive conditions; they do hard physical work on a daily basis with minimal caloric intake. Such a region has been described in the USSR (Georgia) where men not infrequently appear to live to be over 100 years of age, although birthdates are uncertain. It would be of great interest to ascertain whether these aged Georgians fail to develop BPH *or if they have had BPH* whether the nodules regressed after a critical period of aging. Recognizing the difficulties involved, a critical collaborative study of the prostate in this group of Georgian men might provide an answer to whether or not BPH is necessarily an invariant feature of aging in the male. Detente between the USSR and the US at the level of BPH investigation may yet provide real benefits for man, if not mankind.

Q–5: ***Is BPH related to Prostatic Carcinoma?***

Probably not. As Rotkin's review demonstrates, however, we do not really know. Several points are clear. Prostatic cancer primarily involves epithelial elements in the *peripheral* regions of the "prostate proper", hardly ever the periurethral region which is the locus of BPH. The incidence of clinically manifest prostate cancer is much lower than that of BPH. It should be noted that in terms of frequency, carcinoma of the prostate is the most common malignancy in the male; it may be mentioned that many non-metastasizing prostate cancers may be rather asymptomatic. There is no explanation for the curious predilection of the prostate gland to develop tumors (whether benign or malignant) relative to other accessary glands of the male genital tract (seminal vesicles and Cowpers gland)! *What is the special feature of the prostate among the androgen-dependent tissues of the body that makes it the preferred site for pathological growth processes?*

Q–6: ***Is there an analog of BPH in the Female Genital Tract?***

Perhaps. Uterine fibroids, histologically characterized as leiomyomata, are analogous to at least one type of BPH nodule. As with BPH and prostatic cancer, fibroids are benign tumors clearly differentiated from uterine carcinoma which predominately involves epithelial elements. The etiology of uterine fibroids, as with BPH, is completely obscure. However, it is not without interest that in the US, Blacks appear to have a higher incidence of fibroids (and develop them earlier) than non-Mediterranean Caucasians; it will be recalled that there was a tendency for Black males to develop BPH earlier than Caucasians. The incidence of fibroids in women increases with age *up to the point of menopause:* The distinction between the etiologies of a benign tumor and carcinoma, suggestive in the case of the prostate, is more clearly differentiated in the female genital tract. If the underlying hormonal basis for the development of uterine fibroids is similar to that for BPH, it also follows that if men underwent menopause at age 45 to 50 years (and actually stopped secreting their sex-steroids), BPH might be less of a problem than it now represents. If there were a male "menopause", it would be expected to have lesser effect upon the incidence of prostatic cancer.

Q–7: *What is the role of the testis in BPH?*

It is widely accepted that the development of BPH requires the presence of testes. Clinicians agree that if males are castrated before puberty, BPH hardly ever develops later. Men castrated in youth or as young adults have been treated with androgens for many years and BPH has rarely if ever been reported in such cases; the presumption is that if such androgen-treated castrates had indeed developed BPH later in life, these cases would have been reported in the literature. However, Scott mentions as a very unexpected finding the case of a single patient castrated at age 28 who subsequently developed BPH at 60 without having received hormone therapy

It should be emphasized that these views concerning the role of the testes in BPH are clinical impressions, based upon very limited observation involving only a small number of castrated males. Objective information is required concerning the state of the "inner" prostate in a large series of men who have been castrated at various ages in order to

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assess the role of the testes in BPH definitively. *If the single case reported by Scott is not "exceptional" and if prepubertal castrates never have BPH*, then a strong case can be made that the initiation of BPH occurs early in adulthood; the subsequent growth could then be "driven" by androgenic steroids derived from the adrenal cortex or by other forces of a hormonal (or non-hormonal) nature. Study of the prostate in aged castrates who previously had received androgens would likewise be of critical importance. If, as is widely assumed, castrates treated with androgens subsequently fail to develop BPH at age 60 or thereafter, such a finding would provide support for the thesis that the testis contributes "something more" than testosterone to the development of BPH. A number of workers in the BPH field, including Grayhack, have long felt on the basis of several lines of evidence that the testis may be involved (or required) for the production of an unknown ("U") factor critical for the development of BPH.

I do not know whether it is possible to develop a program and a registry system to collect systematically the relevant histological data on the prostate of the large number of men castrated in the US and Europe during the past 30–50 years. If sufficient information on BPH in this group could be obtained and then correlated, such data could be of fundamental importance. The time of onset of BPH and the issue of whether or not a testis "U"-factor is indeed operative in BPH might well be resolved.

Once BPH has developed to a degree where urinary obstruction is present the testes seem less important. If older men with advanced BPH are castrated, relief of bladder neck obstruction symptoms is variable; castration produces regression of epithelial elements but has less (if any) effect on the stromal elements of BPH nodules (Scott). This clinical impression has received strong support from the biomorphometric studies of DeKlerk, Heston and Coffey in the rat prostate, where differential effects of castration and androgen administration upon stromal and epithelial cells are clearly observed. Thus following castration in rats the stromal elements regress very much less than epithelial cells; the response of epithelial and stromal cells to subsequent androgen treatment is likewise different. The important point to be emphasized is that *each cell type in the prostate does not necessarily respond in quantitatively equivalent fashion to androgen manipulation in one direction or another*. Since the cellular composition of human BPH nodules is variable, it is difficult to predict the response to hormone manipulation whether achieved by castration or administration of anti-androgens. To the degree that stromal elements are involved in a BPH nodule, anti-androgens may be less effective in promoting regression of these cells relative to epithelial cells and might predictably fail to provide relief of bladder neck obstruction. The fact that human BPH can grow in organ culture with serum but without addition of further exogenous androgens (Lasnitzki), provides yet another "hint" that attempts to cause regression of stromal BPH nodules using classical anti-androgens (e.g. cyproterone acetate) may have inherent limitations. If growth and maintenance of cultered cells in BPH nodules are indeed independent of physiological concentrations of androgens, how could this be "explained"? Several possibilities come to mind: The simplest is that such cells may have undergone prior transformation in vivo so that BPH cells are no longer androgen dependent; alternatively the cells of BPH may retain a requirement for androgen but develop steroidogenic capacity so that they produce the androgens required. In either case, if human BPH nodules could be successfully transplanted and maintained in animals (as may be the case with the "nude" mice described by Gittes and McClaughlin), such a preparation might well provide a model system to "screen" a large number of chemical agents for "anti-BPH" activity. Precisely which type of compounds should be "screened" is difficult to say. I would think, however, that agents other than conventional anti-androgens should be tested. If we knew the mechanisms involved in the initiation of BPH, as well as its subsequent development, a screening procedure of this type would be useful in any attempt to search rationally for new compounds capable of causing regression of the stromal elements of prostate.

II. Models for Experimental Study of BPH

Experimental model systems for study of various aspects of BPH were discussed at the workshop by several participants. Special attention was given to various attempts which have been made to produce prostatic hyperplasia experimentally in a variety of animal species. The review by Walsh revealed that no animal model faithfully reproduces the gross and microscopic pathological process observed in human BPH; his review raised a fundamental question concerning "models" in experimental investigation which may be put in the following form:

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Q–8: *What should we expect from Animal Models?*

It is generally agreed that the investigation of BPH has been seriously hampered by the absence of a satisfactory animal model where BPH could be produced experimentally. It is felt that the "sought for" model should essentially replicate the picture of human BPH. Dogs spontaneously develop cystic hyperplasia of the prostate upon aging but many remain unconvinced that the disease in the dog is a satisfactory model for human BPH. Thus, the prostate changes which develop in the dog involve the entire gland, and the hyperplasia is primarily *epithelial* and *non-nodular* in sharp contrast to human BPH. Also in contrast to man, prostatic hyperplasia in the dog promptly regresses following castration or testicular suppression (as with pharmacological doses of diethylstilbestrol). Given these and other differences, experimental studies of prostatic hyperplasia in the dog are considered by many to have minimal significance for human BPH.

Similar reservations apply to studies in rodents, where attempts have been made to produce BPH by the administration of estrogens and other steroids. It has long been known that estrogen treatment of male mice produces marked changes in prostate structure (including metaplasia in the anterior lobe and stromal overgrowth) with variable urinary sequellae. These studies in mice suggest the possibility that estrogens might participate together with androgens in the development of human BPH. However, this type of evidence has been generally discounted because of the profound cytological differences observed between estrogen-treated mouse prostate tissue and human BPH (where squamous metaplasia is hardly ever observed).

If one sets the standard that an animal model should faithfully reproduce the gross and microscopic picture found in human BPH, then there are no animal models to study BPH. But this requirement imposes unrealistic constraints upon a model system! Model systems have been successfully employed in biology by sharply focusing on a specific feature of a given complex system and then studying this feature in a simpler model system, ignoring major and often profound differences in other features of the two systems. Insights about the complex systems are obtained by study of the simpler model system, when the underlying mechanisms or principles operative in expression of the feature selected for study are present in both systems. Thus, our present understanding of the principles involved in replication of the genotype in humans is based to a very large extent upon investigation of replication in phage and E. Coli. Study of these simple systems led to concepts which were subsequently extended and found to apply to mammals and man. The *underlying principles of replication*, not the precise mechanistic details, were found to be identical in E. Coli and man. In similar fashion, despite profound differences in the morphology and biochemical characteristics of the prostate gland among different species, study of androgen action in a single model system (rat ventral prostate) has provided a basis for a unitary concept of androgen action, where a specific steroid-receptor complex acts to initiate physiological action. The specific steroid moiety which interacts with an androgen receptor may vary from one tissue to another; the precise hormonal response produced may likewise vary depending upon the specific set of proteins synthesized; the overall pattern, however, is identical in terms of principle. The general concept derived from study of the rat ventral prostate model appears to be pertinent to the prostate gland of all species and to other androgen responsive tissues as well.

If one is concerned with the problem of prostatic hyperplasia, or more specifically *epithelial hyperplasia*, then the dog may well provide a useful model for obtaining insights concerning this aspect of human BPH. If one views *disturbed architectural arrangements of cellular elements* or *overgrowth of stroma* as a central feature of BPH, study of estrogen-treated mice may provide a useful model to study this aspect of BPH. The validity of a model for BPH does not depend upon reproduction of this human disease in all its specific details. The fundamental question is whether a given model and the human disease have underlying similarities, which once revealed by study of the model, can be examined and demonstrated in human BPH.

Consider in this connection, the report of Wilson that long term administration of testosterone or 5α–DHT to castrate dogs *fails* to produce prostatic hyperplasia, whereas dogs with testes intact develop BPH spontaneously upon aging. Wilson's results clearly suggest that a "U" factor, requiring the presence of the testes, is critical to the development of hyperplasia in the dog. Indeed, these dog studies provide the best evidence currently available in support of the view, suggested by some clinicians, that a testes "U" factor is involved in human BPH. The nature of the hypothetical "U" factor is completely obscure. Wilson's preliminary findings that long term administration of 3α-

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androstanediol to castrate dogs *does* produce prostatic hyperplasia (and that this effect appears to be potentiated by estradiol) not only suggests that the "U" factor may be a steroid, but opens new possibilities for understanding human BPH. Do reduced products formed from testosterone and DHT (such as 3α-androstanediol and/or its metabolites) if produced endogenously over a long period of time induce prostate hyperplasia in humans as well as dogs? If hyperplasia is produced does it involve stromal as well as epithelial elements? We do not presently have the answers to these questions. But one point is clear; the dog model which many have regarded as having minimal significance for human BPH, *if properly utilized* appears to provide a basis for a new experimental approach to certain fundamental aspects of human BPH.

III. Alterations in the Circulating levels of Hormones Associated with Aging

The possibility that changes in hormone levels may be involved in the development of BPH was considered during the conference in contributions by Ewing, Vermeulen, Horton, Boyar & Hellman, and McDonald.

Q–9: *What happens to hormone levels with aging?*

The presentations cited converged to give the following picture: The testis undergoes characteristic functional and morphological changes with aging; these changes involve the Leydig cells and cause a decrease in androgen secretion (Ewing). The concentration of circulating testosterone (both "free" and total) is decreased in older men; the circulating concentration of estradiol is less affected. Hence the ratio of "free" estradiol/ "free" testosterone appears to be significantly increased in aged men (Vermeulen). The maintenance of estrogen levels in aged males appears to be accounted for, at least in part, by increased "extra-glandular" aromatization of testosterone and androstendione (McDonald). Thus, it appears that as the male ages, circulating levels of estrogen are maintained while the levels of androgens fall. Associated with these changes in circulating levels of gonadal steroids, LH levels, not unexpectedly, increase (Boyar & Hellman). In elderly men *with BPH*, where the peripheral blood levels of testosterone are low, the 5α-DHT levels may be either normal or increased; Horton suggested that the increased 5α-DHT/ testosterone ratio observed in this group may possibly be significant in relation to BPH. However, it remains to be determined whether the preliminary findings reported are consistently observed in other elderly men with BPH; and, if they are, whether they are related to aging alone or to aging plus BPH.

IV. Cellular and Molecular Mechanisms of Androgen Action in the Prostate:

It has been a fundamental assumption in the BPH field if we really knew *how* androgens act to regulate growth and cell division in the prostate, we would be able to define the specific factors disturbed in BPH and thus be in a position to control this pathological process.

Q–10: *How do steroids act?*

Our present view of androgen action in the prostate is derived from studies undertaken to elucidate the successive molecular events of androgen action in the rat ventral prostate; the results obtained converged with findings obtained with other steroid hormone systems to reveal a pattern of cellular action applicable to all steroid hormones. The issues and facts involved in the general problem of steroid hormone action upon responsive cells in target organs are too complex for any simple summary comment. But one fact can be stated briefly and needs to be stated sharply. It is now a fundamental postulate that the cellular response produced by a steroid hormone is the consequence of a primary action of a specific steroid-receptor complex at the nuclear locus to initiate the following linear sequence: [steroid receptor complex] → DNA → RNA → proteins → "everything else." The selectivity of cells for steroids is ascribed primarily to the selective binding features of a specific steroid receptor protein, mobile in the cytoplasm: the specificity of cellular response is ascribed to the secondary binding of the steroid receptor complex at specific nuclear "acceptor" sites, so that a specific set of genes are activated. For all steroid hormones except the androgens, the active steroid-receptor complex translocated to the nucleus involves the principal circulating hormone (estradiol, progesterone, cortisol, aldosterone). As previously mentioned, in prostate and some androgen target organs, the "active steroid" of the androgen receptor complex is 5α-DHT; in other target organs testosterone may be the active intracellular hormone.

Current research in steroid hormone action has been primarily concerned with the "early steps" in the sequence of hormone action; particular emphasis has been given to the elucidation of the

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molecular nature of the steroid hormone receptors in various model systems and to the mode of receptor-steroid action at the chromatin locus. Less attention has been given to the later steps in the steroid hormone action (the "everything else" of the general sequence), which in the case of androgens and other sex steroid hormones includes DNA replication and cell division. The latest "spectacular" in the field of steroid hormone action is the isolation of a homogeneous progesterone receptor from chick oviduct cytosol by the O'Malley group. This progesterone receptor is a dimer (MW–225,000) built of two non-identical subunits (MW–110,000 and 117,000) each with one progesterone binding unit; this receptor, when occupied, has been reported to act directly on oviduct chromatin to stimulate new RNA chain initiation sites. Since fundamental findings once obtained with one steroid hormone system have been found to apply generally to other steroid hormones, the receptor findings obtained in the chick oviduct-progesterone system may well be applicable to steroid hormone action generally. If this view is correct, the specific androgen receptors in various tissues are expected to be dimers with distinctive "hydrophobic pockets" to provide for the selective binding of one type of steroid as against another.

Q–11: *Are there multiple Androgen-receptors?*

It has long been known that testosterone is not necessarily the most potent androgen in bioassay systems. Thus, in rat prostate 5α-DHT was known to be a more active androgen than testosterone. The explanation for this finding became clear, when it was found that: (a) testosterone upon entering prostate cells is rapidly reduced to 5α-DHT and 5α-androstanediols. (b) 5α-DHT (but not androstanediols) binds with high affinity to a specific "receptor" (designated as B protein by Liao) and (c) the 5α-DHT-receptor complex is transferred to the nucleus where it binds selectively to specific "acceptor sites." In the context of our present understanding of steroid hormone action, 5α-DHT has come to be regarded as the "intracellular hormone" of the prostate cell. The fact that agents (e.g.cyproterone acetate) which inhibit the binding of 5α-DHT to the prostate receptor are potent anti-androgens inhibiting the early effect of testosterone to stimulate *RNA* synthesis, as well as its later effects on DNA synthesis, is perhaps the best evidence that the 5α-DHT-receptor complex does indeed function to initiate hormone action. The available evidence indicates that 5α-DHT receptors are also present in the normal human prostate and BPH nodules (Mainwaring). A specific 5α-DHT receptor protein is present in other androgen sensitive tissues (seminal vesicles, epididymis, preputial glands, etc), together with steroid 5α reductase, and it is assumed that a 5α-DHT-receptor complex is the "trigger" for androgen action in the target cells of these tissues as well. There remain other androgen sensitive tissues (such as the levator ani muscle, uterus and kidney) where steroid 5α-reductase activity is absent or low; the available evidence suggests that the androgen receptor in these cells interacts with testosterone to "trigger" action. In sharp contrast to the forgoing, the 5β-isomer of DHT appears to be an "active metabolite" in the erythropoietic system, and a different type of androgen receptor is suggested. Finally there is evidence for a receptor in vagina which interacts with 3, 17 dihydroxy androstene derivatives.

In effect the evidence indicates that the genome may contain information for the synthesis of a set of androgen receptors, with distinctive structural features, so that each specifically interacts and "envelops" (Liao) different steroid structures, (testosterone, 5α-DHT, 5β-DHT, 3α-androstanediols). The 5α-DHT-receptor complex of the prostate may have different surface properties than the 5β-DHT receptor of the erythropoietic system; thus these receptor complexes may interact with different "acceptor" proteins in the chromatin and activate a different set of genes. In like fashion, a 3α-androstanediol-receptor complex might act at different sites than a 5α-DHT-receptor complex to influence a different set of genes. In view of the finding of Wilson that 3α-androstanediol induces prostate hyperplasia in castrated dogs (whereas 5α-DHT and testosterone are ineffective) the fundamental question is raised whether the altered pattern of macromolecular synthesis associated with prostate hyperplasia involves a new androgen receptor which interacts specifically with 3α-androstanediol and has lesser affinity for 5α-DHT.

Q–12: *What is the nature of the androgen receptor in BPH cells?*

One of the central issues in BPH is to define the nature of the steroid receptors present in the various cell types of the human prostate, particularly the stromal cell types present in BPH. Indeed there is a real question whether all stromal cell types have an androgen receptor. Mainwaring described in some detail the difficulties involved in the study of androgen receptors in BPH nodules, which are

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especially difficult to homogenize because of their fibrous nature. The problem cited perhaps might be resolved by incubation of BPH nodules with collagenase (and other enzymes) in a steroid-free Eagles medium for several hours. The prostate cells isolated might be fractionated and separated into stromal and epithelial elements by various technics. It is also possible, in consequence of the isolation procedure, that the steroid receptors present in these isolated prostate cells would be largely unoccupied. Cells isolated by enzyme treatments are generally easy to lyse with hypotonic media and it should be possible to obtain soluble steroid hormone receptors by gentle homogenization. Characterization of the solubilized steroid receptors derived from stromal as well as epithelial cells would be of decisive importance. Through such studies it would be possible to determine whether or not estradiol receptors as well as androgen receptors other than the normal 5α-DHT receptor are present in the cells which comprise BPH nodules.

Q–13: *How can one study differential effects of androgens and other steroids on various cell types in the prostate gland?*

In various studies of androgen action upon the prostate gland, the simplifying assumption has been made that androgen action in *all* of androgen sensitive cell types is essentially similar; that is to say, the 5α-DHT receptor complex is believed to be the primary element for control of growth and cell division in all prostate cell types. This view serves to direct attention away from the possibility that interactions between stromal and epithelial cells may play a fundamental role in the regulation of coordinated growth (or regression following androgen removal) of the prostate gland. The possibility must be envisaged that certain cell types in the prostate may be androgen-independent; the growth of these cell types may be dependent upon trophic factors released from other cell types which are "nearest neighbors." Although the nature of the factors operative in stromal-epithelial interactions are obscure, the problem raised is fundamental to an understanding of BPH and its medical control.

The biomorphometric procedure described by Coffey and associates, which have clearly shown that epithelial and stromal elements respond differentially to androgen manipulation are clearly a step in the right direction. The specific technics utilized, however are specially laborious and difficult, even at the present level of cell analysis where all non-epithelial cellular elements are considered together as "stromal cells". The effectiveness of the biomorphometric procedure would be considerably enhanced, if one could define distinctive enzyme or protein markers to characterize the specific cell types of the prostate, which might then be used (rather than cytological methods) to determine changes in cell types, associated with steroid manipulation. For example, the enzyme involved in hydroxylation of proline (proline hydroxylase) might serve as the "marker" for the cell type involved in collagen synthesis; myosin (detected by antibody technics) as the "marker" for muscle elements; tartrate-inhibitable acid phosphatase an an enzyme "marker" for epithelial cells. Steroid 5α-reductase enzymes which appear to be present in *all* prostate cells (but this remains to be established) would not qualify as a specific cell "marker." Given such an expansion of the "biomorphometric" approach, the issues posed by Coffey, Scott and others relating to differential effects of steroids upon different cell types in the prostate might well be rapidly resolved.

V. A Summary of Views Concerning the Etiology of BPH

The view has been long established that the primary factor responsible for BPH involves certain specific changes associated with the aging process, and influences on androgen action and/or steroid metabolism in the prostate. The main thrust of the experimental approach to BPH developed from this view is the assumption that if we really had complete information concerning androgen action and steroid metabolism in the normal and aged prostate we would be in position to resolve the problem of BPH. The striking finding that 5α-DHT levels are high in BPH nodules, relative to neighboring "normal" prostate tissue, is one of the major findings in the BPH literature, which lends support to this point of view. The question may be asked: what mechanism(s) is (are) responsible for the increased 5α-DHT levels observed in BPH? Mainwaring made several specific suggestions in this connection: (1) The uptake mechanism for circulating testosterone might be more active in BPH (2) there might be alteration of androgen receptors in BPH, such that affinity for 5α-DHT is increased and 5α-DHT "retention" is increased (3) the "exit" mechanism for DHT might be decreased in BPH. As an additional possibility, it was suggested that prostate production of 5α-DHT from endogenous precursors might be increased in BPH (Horton). The superfusion experiments of Giorgi showing that retention of DHT is much greater in BPH

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nodules than in normal prostate, might be regarded as providing support for (2) or (3). The possibility that agents act at the cell surface as in (1) to facilitate testosterone entry into prostate may serve to relate prolactin to BPH; there is evidence that prolactin binding sites in prostate (Friesen) may represent prolactin receptors, perhaps involved in facilitating testosterone entry (perfusion studies of Resnick). Moreover, there is some evidence that estrogens may increase prolactin secretion; thus the possible participation of estrogens in BPH might be accommodated via this mechanism. Having sketched this possibility, it is fair to state that the idea that prolactin acts to increase androgen entry into prostate cells is regarded, by many, as very debatable.

Once listed, it is possible to study each of these proposed mechanisms systematically in a major program to define the molecular basis for 5α-DHT accumulation in BPH. I have no doubt that such a program would elucidate important features of BPH. However, such a study would not serve to establish whether the increased 5α-DHT levels observed in BPH are *causative* or merely one of many *consequences* of a completely different process that is primarily responsible for BPH. For whatever it is worth, my intuitive "feelings" are that 5α-DHT accumulation is a *consequence* of BPH, *not its cause*. Indeed, to the degree that study of dog BPH mirrors human BPH, Wilson's studies on the effects of 5α-DHT administration to castrated dogs indicated that 5α-DHT accumulation in the prostate *in itself*, is not sufficient to produce prostate hyperplasia. As previously mentioned the idea that a "U" factor (involving the testis) perhaps related to 3α-androstanediol is involved in human BPH received support from these studies on the dog.

If one is prepared to accept the mouse model as having significance for human BPH, the possibility must be seriously entertained that estrogens do play a role in human BPH, particularly with respect to promoting stromal overgrowth. Farnsworth cites evidence that in aged rats, specific estradiol receptors are clearly detectable in the ventral prostate. The findings reported at this conference that the ratio of circulating estrogen/androgen markedly increases with age, are also consistent with the ideas that estradiol may well participate in BPH. Once again, the question is posed, however, whether the changes observed in circulating levels of steroid hormones are the basis of BPH or merely serve to promote the growth of prostate cells altered by other agencies which initiate BPH.

Two of the participants in the workshop directed attention to a variety of factors, independent of steroid hormones, that they felt might be important in the initiation of BPH. Farnsworth and Williams-Ashman independently listed a set of potential causative factors, none of which can be excluded on the basis of our present evidence. Given the complexity of the BPH problem and the multiplicity of factors that may be operative, it is useful to differentiate between the two issues involved in this discussion: (a) what is the causative basis for the initiation of BPH? (b) What are the subsequent factors, hormonal and otherwise, required for the further development and abnormal growth of these nodules. It should be emphasized that the same agencies need not be involved in (a) and (b).

VI. A Speculation Concerning the Etiology of BPH

A theory which purports to account for the etiology of BPH should be able to explain *all* of the central features and established findings in this disease process. I do not have such a theory. Nor am I sure that there is sufficient "hard" data available to develop an all-inclusive theory of this type. Nevertheless, I should like to try to indicate the nature of such a theory by returning to three of the questions previously posed, that I take to represent central issues in BPH.

(a) Why does the prostate in the aging male have a special predeliction for abnormal growth? The other accessory glands of the male genital tract (seminal vesicles, Cowpers gland) as well as other androgen dependent target tissues in the same aged male, are exposed to the same circulating hormones as the prostate. Despite this, these tissues rarely, if ever, develop pathological growth processes.

(b) Why is it that BPH (and prostatic cancer) involves cells in *distinctive regions* of the human prostate?

(c) What is the underlying basis for the disturbed cellular architectural arrangement that appears to be a distinctive feature of the BPH nodule?

Question (a) suggests that the primary defect responsible for abnormal prostate growth relates to "special features" of prostate cells, relative to all of the other androgen-sensitive target cells. These "special features" may reflect intrinsic genetic features of prostate cells; alternatively there may be "special environmental conditions" so that cells in the prostate become vulnerable. The altered pattern of circulating hormones associated with aging

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may play a role in the development of pathological growth in the prostate, (e.g. increase in the ratio of circulating estrogens/androgens), but such a role is likely to be ancillary, not primary. In effect, changes in circulating hormone patterns may be "a necessary but not sufficient condition" for the development of prostate pathology.

If the fundamental problem thus relates to "special features" of prostate cells or to "special conditions" in the prostate, where do we go from here? In their presentations, Williams-Ashman and Farnsworth listed a large number of special agents which might possibly be involved in producing abnormal growth in the prostate; their listing included intrinsic genetic features of prostate cells associated with the aging process, bacterial or viral infections, immunological parameters and several other factors.

Can one focus on some feature of BPH to direct our attention upon the likely "special" agency involved? Question (c) raises the proposition that the *mechanisms of intercellular and intracellular communication normally involved in coordinated growth and cell division of neighboring cells within a tissue are profoundly altered in BPH.* Thus cells grow out and divide without respect for their neighbors. What do we know about the mechanisms normally operative to achieve coordinated growth and division of cells within a tissue? Our present concepts of cell biology recognize cell-cell interactions between various cell types in a tissue (stromal and epithelial cells in the prostate; neuron-glia in brain; mesenchymal cell-parenchymal cell in other tissues); however, the molecular nature of the elements involved in this type of informational transaction between cell types is presently obscure. More information is available concerning the phenomenon of "contact inhibition" (more properly referred to as "density-dependent" growth), where normal cells in culture stop dividing when they make "contact" with other cells. Although a variety of mechanisms have been proposed, one view is that contact achieved via the interaction of specific complementary groups at the surfaces of the two "normal" cells, serves to produce a "message" (e.g. cyclic AMP) which inhibits nuclear DNA synthesis and cell division. Whatever the specific mechanism, "contact inhibition" is lost when cells are "transformed" by oncogenic viruses, many carcinogens, and certain but not all mutagenic agents. These transforming agents are thought to act via irreversible modification of gene activity in such a way that the synthesis and/or incorporation of distinctive "markers" into the surface membrane of the cell is altered. Viruses act by introducing exogenous DNA (or RNA) into the cell which is replicated and becomes integrated into the host's genome; carcinogenic and mutagenic chemicals act by irreversibly altering certain regions of the DNA concerned, more or less directly with the synthesis and/or incorporation of the surface markers into the plasma membrane. It is important to emphasize that the alterations produced in DNA are not "repaired" by a very effective repair mechanism, which corrects many "errors" in DNA replication. Because of their altered surface membranes, transformed cells grow out and "touch" but continue to divide; in effect they are no longer responsive in their neighbors. If transformed cells are transfered from culture into animals, only *some* will go to form tumors; others will be destroyed by the immunological system of the host. Recognizing that there are other "explanations", I should like to propose that the cells in BPH nodules are similar to "transformed" cells, where distinctive surface markers of the plasma membrane have been sufficiently altered so that "contact inhibition is lost," but where the surface membrane remains sufficiently "self" so that it is not attacked and destroyed by the immunological system. Farnsworth cites evidence that the normal surface markers of prostate cells (prostatic tissue-specific antigens) are altered not only in prostatic cancer, where such a finding is not unexpected, but in BPH as well.

To this point, it seems a likely possibility that BPH may involve an agent which induces cell transformation. What kind of agencies might be involved in the transformation of prostate cells? Certain (but not all) viruses; certain (but not all) chemicals with mutagenic activity and most carcinogens would appear to be the likely candidates for cell transformation involved in BPH formation. Question (a) directs our attention to mechanisms which might operate so that agents of this type would act selectively upon prostrate cells, without influencing other androgen-sensitive cells in the seminal vesicles and Cowper glands. The problem of selectivity for prostate cells could be accounted for if the postulated transformation factor is endogenous to the prostate. In this connection, I should like to expand on a theme expressed by Williams-Ashman, in a few terse sentences of his communication. He called attention to the fact that the human prostate is conspicious among the accessory sex glands and other androgen sensitive tissues with respect to one biochemical characteristic; considerable amounts of spermine and related polyamines are produced by the human prostate and these products are dis-

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charged into the seminal plasma during ejaculation. Seminal plasma also contains an enzyme, diamine oxidase (which may be derived from either the seminal vesicles or prostate) which converts spermine and related polyamines into reactive aldehydic products; these polyamine oxidation products spontaneously decompose into reactive cyclized derivatives which are cytotoxic and can react irreversibly with DNA. The implicit question raised by Williams-Ashman is whether polyamine oxidation products might be *endogenous* transforming agents. If diamine oxidase is derived from prostate cells as well as polyamines, it is apparent that an endogenous mutagenic agent might be formed within the prostate itself. This agent might penetrate certain prostate cells and interact with the "right sites" on DNA, so that the cell membrane is altered and contact inhibition is lost. However, the problem of Question (b) remains: how can one achieve selective effects on periurethral cells by this mechanism? One possibility is to assume that the enzyme and polyamines are so localized within the prostate that the formation of the hypothetical transformation agent occurs predominantly in the periurethral region.

Another possibility may be considered, however. A transforming agent, whether present or formed in the urethra, might be transferred back into the prostate *via retrograde diffusion*, where it would act to transform the first prostatic cells encountered, which are in the periurethral region. Once it is postulated that retrograde diffusion of a transforming agent from the urethera back into the prostate is possible, several new possibilities arise. Agents present in the urethra, whether derived from the urinary tract or seminal plasma now become possible candidates as transforming agents. Since the prostate is a common site of infection, bacterial DNA, viral DNA (or RNA) associated with bacterial or viral infections of the urinary tract are potential candidates for initiating BPH. DNA fragments from sperm heads (particularly if modified by polyamine oxidation products or bacterial enzymes) represent another. There is no basis at present for distinguishing between exogenous or endogenous transforming agents. In either case the type of periurethral cells transformed would depend on chance; hence the variability of the specific cell types present in BPH nodules. The important point is that once transformed, the cells would respond uniformly, losing their normal surface markers, so that cell growth and division are no longer regulated and coordinated by "contact inhibition"; with transformation other mechanisms (e.g. stromal-epithelial interactions) operative in normal prostate tissue may likewise be altered. Given appropriate hormone stimulation (which may depend upon androgens with or without estrogens), the transformed cells would be expected to develop eventually through further division into the diverse larger nodules which characterize clinically detectable human BPH.

Since "expression" of the genotype in a transformed cell may be modified at multiple loci, it is possible to envisage differences in the pattern of macromolecular synthesis, involving other elements in addition to cell surface markers. Such transformed cells could have multiple steroid receptors rather than a single 5α-DHT receptor; there may be specific receptors for androstanediols and estradiol, as well for 5α-DHT, in stromal as well as epithelial cells. Binding proteins with high affinity for DHT (which may or may not be receptors) may also be present in such transformed cells. If present, such a DHT binding protein would account for the high level of DHT consistently found in samples of human BPH as well as the findings suggesting that DHT is "avidly retained" in BPH relative to normal prostate tissue. In this context, the increased levels of 5α-DHT found in BPH would be the consequence, not the cause of BPH. If BPH is considered as a problem in irreversible cell transformation, one can go on to "explain" other aspects of BPH.

The fundamental question raised by this speculative exercise is whether cell transformation and loss of "contact inhibition" in vivo is indeed the "key" to BPH. One of the specific suggestions advanced here that polyamines derived from the prostate may play an essential role in the formation of an endogenous transforming agent, which acts to initiates BPH, while attractive, is not fundamental to the argument. Other factors might serve this role. A second point, which perhaps may be fundamental to understanding the distinctive locus of BPH, concerns the idea that retrograde diffusion from the urethra back to the periurethral region of the prostate provides a channel for the selective effects of a transformation factor on periurethral cells. Once retrograde diffusion is admitted, agents unrelated to polyamine oxidation products present in urethra (whether derived from urine or seminal plasma) should be considered as possibly involved in initiating BPH.

It is apparent that the cell transformation hypothesis of BPH initiation does not explain everything concerning prostate pathology. It leaves open the question why prostatic cancer selectively

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involves cells in the peripheral regions of the gland. This is not the place to review what is known about the processes whereby some transformed cells become malignant invasive tumors, while other cells remain benign tumors. It is clear that additional factors must be postulated to account for the distinctive loci as well as for the differential development of a malignant tumor in one case and a benign tumor in the other. Nor is it clear why if cell transformation is indeed produced by endogenous factors, only man and the dog develop prostatic hyperplasia spontaneously, whereas the rat and other species do not exhibit this form of prostate pathology.

Independent of the obvious gaps and deficiencies in the speculative views expressed here, many types of new experiments, not previously considered in the BPH field, now suggest themselves. If BPH is a special case of the more general problems dealing with cell transformation produced by mutagenic agents it should be possible to design straight-forward experiments to test the validity of this idea. When BPH has been studied from this point of view, we will know to what degree the present speculation has contributed to a deeper understanding of the BPH problem.

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