SOME ASPECTS OF TESTICULAR PHYSIOLOGY

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Concepts regarding testicular physiology have changed considerably during recent years and are still in a condition of flux. Considerable knowledge has been gained concerning the testis hormone which causes comb growth when injected into capons. Much of this advance has been the result of investigations by Koch[1] and his associates. This comb-growth-producing substance has been isolated by David, Dingemanse, Freud and Lacqueur[2] and is called testosterone.

A substance, probably a metabolic derivative of testosterone with very similar but less active physiological properties, is found in urine. This has been isolated by Butenandt[3] who has called it androsterone. Urinary extracts containing androsterone have been used in experimental work in this laboratory, and the name "androtin" has been used to designate these extracts. The purpose of this paper is to summarize the evidence which leads us to believe that prostatic hypertrophy may be the result of endocrine imbalance, and that a second testicular hormone exists and is involved in the production of this condition.

Moore[4] has recently discussed the physiology of testosterone thoroughly, and it will therefore be reviewed rather briefly in this paper. Its chief function appears to be the stimulation of growth and maintenance of the whole male fecundatory mechanism. Recently, it has been demonstrated by Walsh, Cuyler and McCullagh[5] that this is true not only for the secondary sex glands but for the testes themselves. Following hypophysectomy, complete atrophy of the primary and secondary sex glands always occurs. This atrophy commences three or four days following hypophysectomy, and the changes are almost complete in twenty days. It was found that the daily injection of nine bird units of androtin into hypophysectomized rats over a period of twenty days maintained the testes, seminal vesicles, and the prostate glands of these experimental animals in a condition which could not be distinguished from the normal. The tubules of the testes and the epididymides of the hypophysectomized animals treated with androtin contained more spermatozoa than did the normal controls.

The androtin used in these experiments was made by the continuous extraction of urine with carbon tetrachloride. The carbon tetrachloride was removed by distillation and the extracts purified by distribution between fat solvents. The extract was eventually dissolved in sesame oil and heated in the autoclave at 120 degrees Centigrade for one-half hour. We thus feel that it was not contaminated with pituitary-like

hormone which is occasionally found in the urine of men, and which acts as a gametogenic substance.

Testis hormone has many functions other than the maintenance of the sex mechanism. The most noticeable is that of the maintenance of the secondary sex characteristics. The growth and distribution of body hair is influenced by testicular function. In birds, the plumage and comb and wattles show normal male characteristics only when the testis hormone is present. The distribution of body fat in man and in animals is altered by castration. Due to the fact that epiphyseal closure is delayed, skeletal proportions are abnormal in the absence of testis function—the long bones being relatively longer in a eunuch than in a normal man.

Clinicians have known for many years that there is a close relationship between the adrenals and the sex glands. Hirsutism, virilism and pubertas praecox have frequently been known to be associated with adrenal tumors. Following castration in animals, there is a definite hypertrophy of the adrenals. That this hypertrophy is due to absence of the testis hormone is shown by the fact that it can be controlled by the injection of androtin.[6]

There is ample evidence to prove that testis hormone exerts a profound influence over the pituitary gland. Following castration, a marked change in the histologic appearance of the pituitary glands occurs. The percentage of basophil cells rapidly increases, and some of these basophil cells become vacuolated and finally form signet ring cells—the so-called castration cells of the pituitary gland. Reese and McQueen-Williams[7] claim to have demonstrated that in the castrated rat, basophil cell formation can be prevented or considerably modified by the administration of 4.4 bird units of testicular extract. Nelson[8] failed to control the pituitary glands of castrated male rats with 7.5 bird units of testicular extract daily, although the same dose prevented formation of basophil cells in gonadectomized females.

We have performed another series of experiments which demonstrate that androtin has a marked influence over the pituitary gland.[9] When two rats are united surgically in parabiotic union so that their peritoneal cavities are continuous, diffusible water-soluble substances will pass from one animal to the other. The gonadotropic hormone of the anterior lobe of the pituitary gland is a substance of this type. If one of a pair of parabiotic animals is castrated, its pituitary gland becomes hyperactive as evidenced by the fact that its parabiotic partner shows every sign of pituitary stimulation. If this normal partner is a male, the excessive amount of pituitary hormone coming from the castrated partner stimulates the testes of the normal partner to the production of large quantities of testosterone with the result that its prostate and

seminal vesicles become hypertrophic. We found that if sufficient androtin is injected into the castrated partner to prevent its prostate from becoming atrophic, none of the signs of pituitary hyperactivity are observed in the normal animal.

With some of these and with other facts in view, Moore[10] postulated a theory of pituitary-gonadal interrelationship. According to this theory, the gonadal-pituitary mechanism is self-regulatory. If the testes produce insufficient quantities of testosterone, the pituitary gland is not held in proper control, and it produces larger amounts of gonadotropic hormone. In that manner, the pituitary gland stimulates the testes to the production of the proper amount of testosterone. Conversely, if excessive amounts of testosterone are produced, pituitary depression results and in turn, testicular function is retarded. Moore[11] has also discussed the possibility that two hormones may be elaborated in the testes. If this proves to be the case, his theory of pituitary-gonadal interrelationship will have to be extended to include the second endocrine product of the testes.

Our investigations concerning androtin commenced in 1930 when a considerable quantity of this substance was made from urine in order to treat patients who suffered from hypogonadism. The androtin thus prepared for this study was assayed by injection into capons and also by injection into castrated rats. Each rat was sacrificed and carefully examined at the completion of the test, and it soon became apparent that a castrated rat could not be maintained in a completely normal condition by the use of this hormone.[6] Following castration, the secondary sex glands normally undergo complete atrophy, and the adrenals and the pituitary gland become hypertrophied. The administration of androtin will prevent atrophy of the secondary sex glands in castrated animals and will prevent hypertrophy of the adrenals but will not prevent the pituitary gland from becoming enlarged. Following our publication, Korenchevsky[12] made the same observation regarding the effects of androtin and more recently has repeated these observations using a synthetic comb-growth-producing material. From this, we deduced that there might possibly be a second testicular hormone, one property of which is to control the activity of the pituitary gland. For this hormone, the name "inhibin" has been proposed.[13]

Following this observation that androtin did not control the pituitary hypertrophy which followed castration, we investigated the literature to see whether other workers had discovered anything which might throw further light on the problem of the duality of endocrine function of the testes. A considerable quantity of information on the subject was already available, and other authors who had worked from entirely

different angles had reached the conclusion that the testes produced two hormones.

In 1923, Mottram and Cramer[14] studied the effect of irradiation on the rat and made a very significant observation. They noticed that following irradiation of the testes with roentgen rays, the tubular elements rapidly underwent degeneration and the interstitial elements remained normal. Accompanying these changes in the testes, there was a definite change in the pituitary gland which simulated that which follows castration. They found at the same time, that no atrophy of the secondary sex glands occurred. They concluded that one testicular mechanism controls the pituitary gland while another is responsible for the maintenance of the secondary sex glands.

Johnston,[15] working in collaboration with Dr. Lower, noticed that following irradiation of rats' testes and destruction of the tubular elements, prostatic hypertrophy resulted in some instances. This could be interpreted as being the result of destruction by the x-ray of the inhibin-producing mechanism of the testes, and prostatic hypertrophy would result from pituitary stimulation.

In 1926, Nukariya[16] studied the influence of saline extracts of testes on the pituitary glands of castrated animals and noted that these extracts altered the metabolic processes of the castrated animals but did not alter the number of basophil cells in the pituitary gland following castration. Only a very small amount of testosterone is found in the testes and from a quantitative point of view, one would not expect any effect from the administration of this hormone in a saline extract, particularly when one considers that it is not soluble in saline. This would indicate that there is a water-soluble hormone in the testes which influences pituitary activity. Lehmann[17] states that the injection of water-soluble testicular hormone into castrated rats will prevent the occurrence of histologic changes in the pituitary gland. Martins[18] has more recently studied the same problem and also claims that these aqueous extracts suppress the production of basophil cells in the pituitary glands of castrated rats. Witschi, Levine and Hill[19] experimented with rats which had received roentgen irradiation to the testes. They found that the tubules of the testes were destroyed while the interstitial elements were intact. The secondary sex glands were hypertrophied which showed that the testes were producing excessive quantities of testosterone. Using the parabiotic method, they demonstrated that these animals had hyperactive pituitary glands which, according to our interpretation, means that the inhibin producing properties of the testes had been destroyed, whereas the testosterone producing areas were intact as evidenced by the hypertrophied secondary sex glands.

Evans and Simpson[20] studied the effect of castration and cryptorchidism on the quantity of gonadotropic hormone in the pituitary gland. Following castration, the pituitary gland not only increases in size, but there is an increase in hormone content. The changes which take place in the testes in cryptorchidism are similar to those which follow roentgen irradiation. There is rapid degeneration of the tubular elements during the first month, whereas the interstitial cells persist for a considerable period. Following destruction of the tubular elements, but before the testes lose their power to elaborate testosterone, a definite change occurs in the pituitary gland. The change which occurs during cryptorchidism is similar to that which follows castration in that the pituitary glands of cryptorchid animals also contain a greater quantity of gonadotropic hormone than that in normal animals. In other words, the cryptorchid testis elaborates sufficient testosterone to maintain the secondary sex glands, but the mechanism which controls pituitary activity has failed.

Probably the most striking results obtained in the study of inhibin were those of Martins and his collaborators.[18] In their experiments with parabiotic rats, they attached a castrated animal to either a normal male or female. Following castration, the normal animal was influenced by the hyperactive pituitary gland of the castrated partner. These workers showed that when small quantities of testicular mush from either mature or immature animals were injected into the castrated animal, pituitary hyperactivity was prevented, and they pointed out that this suggested the presence of a second testicular hormone. McCullagh and Walsh[9] have confirmed the experiments of Martins and Rocha using adult male rats and in addition have demonstrated that the injection of aqueous extracts of testes into normal animals will decrease the pituitary activity and therefore produce prostatic atrophy. Myers[21] and his coworkers state that feeding desiccated testicular material to rats caused prostatic atrophy. We repeated his work and obtained apparently confirmatory results. More recently, however, we have had difficulty in duplicating these experiments.

Schrire and Zwarenstein[22] have studied the influence of the testes on creatinine metabolism in rabbits. They state that when aqueous extracts of testes are injected into castrated rabbits, the otherwise abnormally high daily output of urinary creatinine is diminished. Testicular preparations similar to those used by Professor Koch also have the same property; however, as stated above, one does not expect aqueous saline extract to contain more than insignificant traces of testosterone. It would seem to this author that these experiments indicate that inhibin may have some influence on creatinine metabolism. This problem is now being investigated here.

Recently reported experiments by Stein[23] on castrated immature rats indicate that the testes show signs of inhibin production long before there is any indication of a production of testosterone. If the animals are castrated when seven days of age, very marked pituitary hypertrophy occurs in two or three weeks. This is particularly interesting in that the castration of the adult rat frequently does not result in definite hypertrophy in less than two or three months. Hence, the pituitary of the immature animal is more sensitive to withdrawal of inhibin than is that of the adult animal.

There can be no doubt but that the experiments herein reported definitely indicate the production in the testes of the hormone inhibin. It has been our experience, however, that it is extremely difficult to obtain constant results using the techniques so far developed for the demonstration of this hormone. The problem is obviously in its infancy. Nearly all the work so far reported should be repeated and confirmed, and a method of assay must be developed before the work can progress in the proper quantitative fashion.

Clinical Considerations: McCullagh, McCullagh and Hicken[24] studied the effects of the administration of androtin to men suffering from hypogonadism. An insufficient amount of hormone was available to treat these patients for a long period of time; however, there seemed to be definite reasons to believe that the secondary sex glands in man could be stimulated by the use of androtin. In some instances after treatment, motile spermatoza were found in fluid obtained by prostatic massage in cases where none were found before. This is in accord with experimental findings in which androtin maintained the spermatogenic elements in hypophysectomized rats.

Other authors have not mentioned the great clinical significance of the hormone inhibin. The theory of dual endocrine activity of the testes offers an excellent explanation for prostatic hypertrophy in man. If the cells which produce inhibin fail before those which produce testosterone, one would expect hyperactivity of the pituitary gland to follow. The resultant excessive gonadotropic activity in the pituitary gland would cause stimulation of the cells of the testes which produce testosterone. It is known that testosterone is capable of producing prostatic enlargement in experimental animals, and there is no reason to believe that this may not also occur in man.

There are two facts which strongly support this theory of the etiology of benign prostatic hypertrophy. First, it has been known for years that castration in some cases will cure benign prostatic hypertrophy in man. This indicates that the condition is related to testicular activity. Moreover, several castrated patients who have been examined in this

institution have all been found to have atrophic prostate glands. Second, pituitary overactivity in benign prostatic hypertrophy has been demonstrated by Dr. E. P. McCullagh and Mr. Kenneth Cuyler in several patients suffering from prostatic hypertrophy. This has been so definite that a positive Friedman test of the same type as that which occurs during pregnancy has been observed.

The clinical application of this theory has been studied by Dr. William E. Lower who also has collaborated in the experimental work. Dr. Lower has reported his clinical findings in this issue of the Quarterly.

References

1. Gallagher, T. F. and Koch, F. C.: Biochemical studies on the male hormone as obtained from urine, Endocrinology, 18:107-112, 1934.
2. David, K., Dingemanse, E., Freud, F. and Laqueur, Ernst: Über krystallinisches männliches Hormon aus Hoden (Testosterone) wirksamer als aus Harn oder aus Cholesterin bereitetes Androsteron, Ztschr. f. physiol. Chemie, 233:281-282, 1935.
3. Butenandt, A.: Über die chemische Untersuchung der Sexual-hormone, Ztschr. angew. Chem., 44:905, 1931.
4. Moore, Carl R.: The testis hormone, J.A.M.A., 104:1405-1411, 1935.
5. Walsh, Eugene L., Cuyler, Kenneth W. and McCullagh, D. Roy: The physiologic maintenance of the male sex glands, Am. J. Phys., 107:508-512, 1934.
6. McCullagh, D. Roy and Walsh, Eugene L.: Further studies concerning testicular hormone, Proc. Soc. Exper. Biol. & Med., 31:678-680, 1934.
7. Reese, J. D. and McQueen-Williams, M.: Prevention of "castration cells" in the anterior pituitary of the male rat by administration of the male sex hormone, Am. J. Phys., 101:239-245, 1932.
8. Nelson, W. O.: The effect of large amounts of gonad-hormones upon the anterior hypophyses of normal and gonadectomized rats, Anat. Rec., 55:70, 1932-1933.
9. McCullagh, D. Roy and Walsh, Eugene L.: Experimental hypertrophy and atrophy of the prostate gland, Endocrinology, 19:466-479, 1935.
10. Moore, C. R. and Price, D.: Gonad hormone functions, and reciprocal influence between gonads and hypophysis with its bearing on the problem of sex hormone antagonism, Am. J. Anat., 50:13-71, 1932.
11. Moore, C. R.: Sex and internal secretions, Allen, Baltimore, Williams & Wilkins, 1932.
12. Korenchevsky, Z.: Effects produced on rats by synthetic androsteron, Nature, 135:434, 1935.
13. McCullagh, D. Roy: Dual endocrine activity of the testes, Science, 76:19-20, 1932.
14. Mottram, J. C. and Cramer, W.: On the general effects of exposure to radium on metabolism and tumor growth in the rat and the special effects on testes and pituitary, Quart. J. Exper. Physiol., 13:209-226, 1923.
15. Johnston, R. L.: Studies in the physiology of the prostate gland, Endocrinology, 18:123-141, 1934.
16. Nukariya, S.: Über die Bedeutung der Rückresorption des Spermas (auf Grund von Spermainjektion an Kastraten) und über mikroskopische Veränderungen der Hypophyse an jungkastrierten weissen Ratten, Pflüger's Arch. f. d. ges. Physiol., 214:697-720, 1926.
17. Lehmann. J.: Zur frage der Geschlechtsspezifität der Keimdrüseninkrete, Pflüger's Arch. f. d. ges. Physiol., 216:729-748, 1927.
18. Martins, T. and Rocha, A.: The regulation of the hypophysis by the testicle and some problems of sexual dynamics, Endocrinology, 15:421-434, 1931.

19. Witschi, E., Levine, W. T. and Hill, R. T.: Endocrine reactions of x-ray sterilized males, Proc. Soc. Exper. Biol. & Med., 29:1024-1026, (May) 1932.

20. Evans, H. M. and Simpson, M. E.: A comparison of anterior hypophyseal implants from normal and gonadectomized animals with reference to their capacity to stimulate the immature ovary, Am. J. Physiol., 89:371-374, 1929.

21. Myers, H. B., Vidgoff, B. and Hunter, W. C.: Effect of desiccated beef testis upon sex glands of the rat, Proc. Soc. Exper. Biol. & Med., 30:1099-1102, 1933.

22. Schrire, I. and Zwarenstein, H.: The influence of gonads on protein metabolism. II. The effect of injections of saline suspensions of testes and testicular extractions on urinary creatinine after castration, Biochem. J., 26:1886-1889, 1932.

23. Stein, Kathryn F.: A sex difference in gonad-stimulating potency of young gonadectomized rats, Proc. Soc. Exper. Biol. & Med., 33:95-97, 1935.

24. McCullagh, E. Perry, McCullagh, D. Doy and Hicken, N. Fred: Diagnosis and treatment of hypogonadism in the male, Endocrinology, 17:49-63,1933.