THE VITAMINS AND SEX HORMONES CONCERNED IN REPRODUCTION

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The vitamins, the hormones, and the enzymes constitute three important groups of substances of special interest to the biological chemist. Although many of the more startling discoveries in these fields seem already to have been made, our knowledge remains exceedingly elementary. Little is known about the details of the mechanism by means of which these substances are effective, and only recently has any considerable interest been evinced concerning their interrelationship. In a recent review, Bernheim[1] mentioned several interesting examples of the interaction of vitamins and cell catalysts.

The purpose of the present review is to summarize some of the effects of nutritional states on the reproductive organs, and in some cases, to compare and contrast endocrine and vitamin deficiencies. Most of the available information has been the result of work with the rat, and some of the facts presented here may not be applicable to man. Mason[2] recently considered this subject in a comprehensive review, and the reader is referred to his extensive bibliography.

The hormones and the vitamins are closely related in many respects, and both words have been used so extensively that their meanings are no longer very closely defined. For the purpose of this discussion, both may be considered as organic substances necessary for normal health; the vitamins are exogenous, whereas the hormones are endogenous. Depending upon the biological system being considered, some substances may fall into either group. For example, thiamin (vitamin B_1) must be considered as a hormone in plants and as a vitamin in animals. In man, hexuronic acid (vitamin C) is an essential exogenous substance and hence a vitamin, whereas in the rat it is made within the tissues and would therefore be a hormone.

THE TESTES

The male gonads are known to serve two functions: (1) spermatogenesis and (2) the production of one or more hormones. It is also known that both of these functions cease following hypophysectomy and that they can be maintained by injections of extracts of the pituitary gland. Some investigators believe that there are two pituitary gonadotropic hormones, one stimulating spermatogenesis and the other stimulating the interstitial cells of the testes to the production of androgenic material. The androgens or male hormones, in turn, play a role in the

maintenance of the normal anatomy and function of the penis and secondary sex glands, and male sex characteristics. They also probably serve some function in stimulating spermatogenesis, since spermatogenesis continues after hypophysectomy if androgens are supplied. The androgens also have numerous profound metabolic effects.

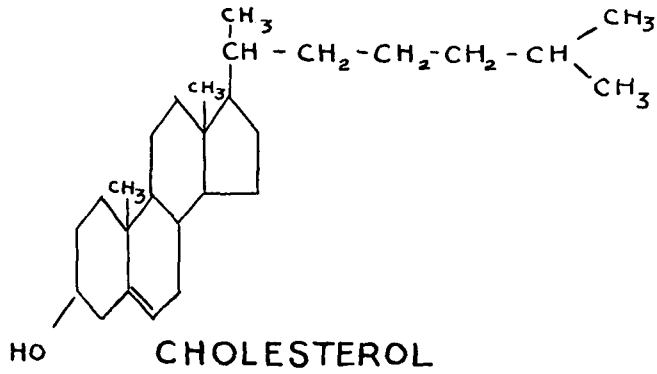
The male hormonal mechanism is controlled in such a way that the production of the various hormones is very flexible and the testes will function normally under a great variety of conditions. The flexibility is attained through a delicately balanced reciprocal relationship between the testes and the pituitary gland. The testes depress the pituitary gland and the latter stimulates the testes. Therefore, hypofunction of the testes results in increased pituitary activity and hence a return to normal. However, deficiencies of certain vitamins will completely disturb testicular function.

Vitamin A, which is a fat soluble carotinoid substance, and the lack of which results in poor growth, xerophthalmia, night blindness, etc., is very essential for proper testicular function. In the vitamin A deficient animal, spermatogenic activity is greatly reduced. In mild deficiency, spermatozoa are not produced, but several layers of spermatogonia and spermatocytes may exist. In extreme deficiency, only a few germ cells remain and the interstitial cells remain almost normal. In the rat, the degeneration of the testes which occurs after severe vitamin A deficiency can readily be cured in a period of about two months. This cure can be effected if adequate vitamin is added to even a very low caloric diet.

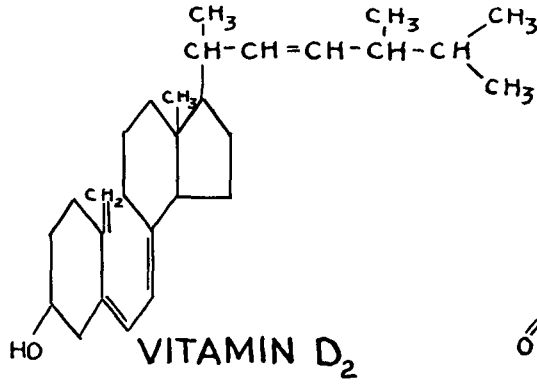
Until further researches have been accomplished, the *vitamin B complex*, in most cases, will have to be considered as a whole. Vitamin B_1 is necessary for testicular function in birds. Studies in the pigeon and the domestic fowl demonstrate profound testicular degeneration in vitamin B deficiency. This is not the result of inanition and can be cured by adding vitamin B to the diet for a period of less than a month. The picture is definitely different in mammals in that in vitamin B deficiency the testes remain almost normal. Any changes which occur can be attributed to loss of appetite with resulting inanition. Chinese coolies have been reported dying of beriberi without any considerably injury to the testes.

It has not been clearly demonstrated whether *vitamin C* deficiency directly influences testis function. The general condition of the scorbutic animal or man, of course, renders him less capable of reproductive activity. Normally, there is a high concentration of vitamin C in the testes, as is the case in other glandular material. However, it seems to have no direct relationship to the hormones in male or female animals.

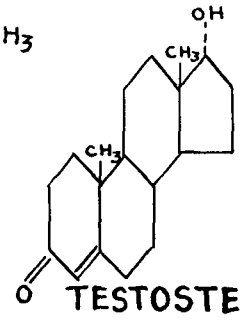
Vitamin D, the antirachitic hormone, is chemically closely related to cholesterol, as are the androgens, the estrogens, and progesterone. It is not impossible that cholesterol is the precursor of vitamin D and also



CHOLESTEROL



VITAMIN D_2



TESTOSTERONE

of the hormones mentioned above. The structural formulas given in figure 1 demonstrate the close relationship between cholesterol, vitamin D_2, and testosterone. Certain of the adrenal hormones and the ovarian hormones are also of this steroid configuration.

It is unnecessary here to review the relationship of vitamin D to the metabolism of calcium and phosphorus. That normal calcification will not occur without this vitamin is well established. Descriptions of the disturbances of mineral metabolism and of the rickety skeleton are available in many standard works. Although vitamin D deficiency does not appear to cause any testicular injury, this vitamin is related in function to the testes in that the androgens also influence bone growth. Following castration or in hypogonadism from other causes, skeletal calcification is normal insofar as calcium deposition and bone composition is concerned. However, normal skeletal maturation does not occur, as evidenced by delayed epiphyseal closure. As a result, the long bones usually continue to grow for an abnormally long period. In the human this results in an individual whose arms and legs are long in proportion to his torso.

Vitamin E: The existence of this vitamin has been known for nearly twenty years, and it recently has been shown to be alphatocopherol. In vitamin E deficiency is seen the most profound testicular tubular damage producable by any nutritional deficiency. At the same time the interstitial cells remain intact and the experimental animals remain vigorous without other untoward symptoms. If the deficiency is mild, the only noticeable symptom may be sterility. As the deficiency becomes more severe, the sperms change in morphology and staining characteristics and eventually disappear from the semen. In complete deficiency, the germinal epithelium gradually undergoes lysis and other forms of degenerative changes until it liquefies or is sloughed off into the epididymis. The tubular content is then limited to a syncytial Sertoli mass. These histologic changes are usually accompanied by loss of sex interest.

In the rat the testicular damage seen in vitamin E deficiency differs from other similar changes, in that it not only is more profound but also is completely irreparable. Once the rat is depleted of vitamin E, and before morphologic changes have more than commenced, the addition of vitamin E to the diet will not prevent destruction, nor will it produce a return of spermatogenesis.

The reaction of the rat to vitamin E deficiency is of considerable importance in interpreting the endocrinologic findings concerning testis-pituitary interrelationships. Following castration, the pituitary gland undergoes certain functional and morphologic changes. The basophilic cells, in particular, are altered and the gonadotropic potency of the whole gland increases, as indicated by assay of the glandular material using immature animals. Very similar, if not identical, changes appear in the pituitary glands of vitamin E deficient animals. In vitamin E deficiency the interstitial cells which produce the male sex hormone, are morphologically and functionally intact, as indicated by the condition of the secondary sex glands. It is possible, therefore, to argue that in both vitamin E deficiency and castration the changes in the pituitary gland are the result of destruction or removal of the tubular portion of the testes. These, and many other considerations, have led to the postulation of a testicular hormone other than the androgens. Indeed, several workers have effectively used nonandrogenic testicular extracts to prevent the castration changes in the pituitary gland. The hypothetical pituitary hormone which is thought to depress the pituitary gland is known as inhibin. In this laboratory large quantities of testicular concentrates which act as sexual depressants have been made. Attempts to isolate inhibin are now in progress.

The picture of testicular physiology depicted above, however, is complicated by certain known facts. Among these is that several of the androgens (testosterone, testosterone propionate, and dehydro-

androsterone) have been definitely shown to maintain normal function and morphology in the pituitary gland of the castrated rat. Also, the testes are known to contain estrogens, which also have a direct effect upon the pituitary gland. It therefore becomes evident that further study is required completely to elucidate these problems.

Inanition: Generally in starvation the testes of the young fail to develop in regard to both spermatogenic and androgenic properties. Except in extreme conditions of starvation, the spermatogonia remain normal in appearance and apparently in function. The spermatocytes, however, undergo complete degeneration. Unless vitamin E deficiency exists, a return to normal occurs if the animal is placed on an adequate diet.

In the adult, some loss of weight can occur without testicular injury. However, severe weight loss results in a return of the testes to a condition similar to the prepuberal state. The germinal epithelium continues to proliferate but the germ cells fail to mature. The interstitial elements appear normal, but do not secrete the usual amount of male sex hormone. These changes can at least partially be attributed to failure of the pituitary gland adequately to stimulate the testes. The adult testis recovers readily from the effects of inanition when the cause is removed.

ACCESSORY SEX GLANDS

From the endocrinologic standpoint, the secondary sex glands of the male are dependent upon the testes. Under normal conditions their activity is controlled by the amount of androgen available. Dietary deficiencies not only alter the amount of androgen available, but also have a direct effect upon the secondary sex glands. These glands are lined with an epithelial membrane. They are therefore susceptible to keratinization in vitamin A deficiency. Such keratinization has been reported in the glandular structures as well as in the epididymis and vas deferens. Infection of these structures probably occurs more readily under these circumstances. Also, the filling and stoppage of the efferent ducts with cell debris probably seriously interferes with their function.

Vitamin A has a marked growth promoting effect. In its absence the secondary sex glands become atrophic. This atrophy results from testicular failure, as evidenced by the fact that testicular stimulation by gonadotropic substances will restore the glands to the normal state. Similarly, in vitamin B deficiency, it appears that the pituitary-testis mechanism is responsible for the atrophy of the secondary sex glands. If the testes are stimulated to produce androgens, the seminal vesicles and prostate do not show pathologic changes.

Vitamins C, D and E have little, if any, influence on the secondary sex glands.

THE OVARIES

As with the testes, the ovaries are functionally reciprocally related to the pituitary gland. Ovulation and the production of ovarian hormones (estrogens and progesterone) occur as the result of pituitary stimulation. The gonadotropic activity of the pituitary, in turn, is governed to a considerable extent by ovarian hormones. Thus, in many respects, the ovaries are analogous to the testes. Their reaction to vitamin deficiency, however, is quite different in that they are much less susceptible than are the testes to nutritional deficiency.

Deficiencies of vitamins A, B, C, D, and E seem to have no direct effect upon the ovaries. Whenever functional or morphologic changes occur in the ovaries because of nutritional disturbances, these changes probably are due to anorexia and cachexia resulting in malnutrition, decreased production of sex hormones by the pituitary gland and, in turn, by the ovaries.

As in the case of the male, there is considerable evidence to demonstrate a relationship between the female gonads and calcium metabolism. The close chemical relationship between the sex hormones and vitamin D has been pointed out. Whether or not it is this similarity that results in effects on calcium metabolism is not known.

Although the ovary is singularly resistant to vitamin deficiencies, it is peculiarly susceptible to inanition. In young animals, starvation causes marked retardation of sexual development with failure of follicular maturation. In the adult in all mammals studied, ovulation is repressed by a degree of inanition which has little effect upon spermatogenesis. Cessation of cyclic activity and sterility occurs during periods of starvation.

THE UTERUS AND VAGINA

The development of the vagina and uterus, and the cyclic changes which occur in the vaginal epithelium and in the endometrium are influenced by estrogens and progesterone which are products of the ovaries. These organs are, however, more sensitive to vitamin A deficiency than is the ovary. The epithelial keratinization which occurs in vitamin A deficiency is observed in both the uterus and the vagina. In the uterus this may cause increased susceptibility to infection and decreased fertility. The latter may be the result of difficulty in the ascent of the sperm or of improper conditions for nidation.

In vitamin A deficiency the keratinization of the vaginal epithelium results in the appearance of cornified epithelial cells in the vaginal smears. Such a smear could be misinterpreted as indicating continuous estrous resulting from estrogenic stimulation. This is clearly not the case, since it occurs also in oöphorectomized animals. The vagina responds very rapidly to vitamin therapy and becomes normal in the course of a few days. Hypervitaminosis-A has been observed to produce vaginal symptoms characterized by a prolonged period of di-estrous, during which there is failure of epithelial cornification.

None of the other vitamins have been demonstrated to cause a direct effect on the vagina or the nongravid uterus. As will be shown, vitamin E influences gestation, and any factor (e.g. vitamin B deficiency) which produces inanition may indirectly effect these organs. The atrophy and degeneration which occurs in all the tissues of the vagina and uterus during starvation are readily reparable by dietary measures.

GESTATION

The epithelial changes induced in the uterus in the vitamin A-deficient animal results in serious impairment of reproductive function. As stated by Mason[2], "pregnancy usually culminates in variable degrees of fetal resorption, late fetal death, extended gestation, prolonged and difficult parturition, frequent stillbirth, and high mortality of the viable offspring." None of these reproductive defects appears to be the result of any interference with the hormonal mechanisms involved. In every instance, the gestational failure seems to be caused by the pathologic changes in the epithelial tissues which results in failure of proper function or increased susceptibility to infection.

It has already been pointed out that pituitary-gonadal function is greatly depressed during vitamin B deficiency, and that the effect of hypovitaminosis-B on the reproductive system is probably nonspecific and is the result of inanition. These statements also apply to the influence of vitamin B deficiency during gestation. Dead or puny offspring frequently result. The fetus usually is small, which may reflect the absence of growth hormone. In severe deficiency resorption of the fetus or abortion may occur. Sometimes frank beriberi may occur during gestation because of added requirements for thiamin. In this connection it might be mentioned that during lactation thiamin requirements are very high, and that as a result vitamin B_1 deficiency may readily result if the diet is not consistently high in that vitamin.

Vitamin C deficiency will interrupt pregnancy or result in the birth of offspring with scorbutic tissues. Some investigators believe that during pregnancy the symptoms of scurvy are relieved somewhat. Whether

or not this is due to a metabolic change or to vitamin production by the fetus has not been determined.

The reproductive capacity is retained in an amazingly normal fashion in vitamin D deficiency. The fetus is less apt to show rickets than the mother to have osteomalacia.

The female rat on a vitamin E free diet shows no demonstrable changes in reproductive capacity until after the onset of pregnancy. Pregnancy is terminated by the uterine resorption of the fetuses. The pathology of the process has been studied in detail and is unique. It consists essentially in a failure to establish adequate fetal circulation through the placenta. Considerable evidence indicates that vitamin E is essential in most mammals. In the human it may play some role involving the metabolism of the estrogens during pregnancy. It has been reported to be of value in spontaneous abortion.

CONCLUSION

In considering the physiology or pathology of the reproductive organs, it is clear that the normal endocrinologic control can seriously be disturbed by numerous nutritional deficiencies.

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