Histology. — *The influence of Hormones on the Os Priapi.* By S. T. BOK, S. E. DE JONGH and I. VAN ZWANENBERG. (From the Histological and the Pharmacological Laboratories of the University of Leiden. Directors: Prof. Dr. S. T. BOK and Prof. Dr. S. E. DE JONGH.) (Communicated by Prof. J. VAN DER HOEVE.)

(Communicated at the meeting of November 28, 1942.)

In a previous paper [1]) we pointed to the following facts: Testosterone, when administered for 4 weeks to castrate immature rats induces a strong development of the os priapi. In the hypophysectomized castrate testosterone causes an enlargement, comparable with the foregoing, but the differentiation is less distinct. A difference exists between hypophysectomized and intact *un*treated castrates in view of loss, resp. persistance of the cartilage capsule on the proximal end.

Two questions remained still unanswered:

1. Is the difference, in view of the development of the os priapi between hypophysectomized and intact castrates, treated with testosterone, essential or is it but a question of tempo?

2. Is the influence, that appears to be exerted by the hypophysis, due to growth hormone?

We tried to solve the former question by enriching the already available material with non-hypophysectiomized castrates, treated with testosterone (in stead of for 4 weeks!) resp. during 1, 2 and 3 weeks. We then investigated, if we could find in the thus obtained series a place for the hypophysectomized animals treated for 4 weeks.

The second question gave rise to experiments, in which hypophysectomized castrates received, besides testosterone, growth hormone, in order to realize an, albeit perhaps partial, substitution of the hypophysis.

We are indepted to Prof. Dr. E. LAQUEUR and Dr. ELIZABETH DINGEMANSE, who suplied us with growth hormone, a rather pure preparation, containing 1 **R U** per 50 γ.

The new experimental material is recorded in table I and II. The histological description as well as the comparison with the already available microscopical preparations (see previous article) gave rise to the following notations.

Normal architecture.

In young rats (as had been used in these experiments) the penis skeleton consists in 1. the os priapi and 2. a solid body, situated in the middle of the glans, distally and somewhat dorsally of the os priapi.

The os priapi has the shape of a round-headed nail: its longest part, the shaft, the axis of which runs parallel to the urethra, has a fairly constant width, its proximal end is broadened: the head. In the shaft the medullary cavity is narrow, its diameter measuring about 0.1 of the total diameter of the shaft, in the head it is wide, the wall (compacta) of the head being scarcely thicker than that of the shaft. The cavity contains wide mashed reticular connective tissue, no bone rods (trabeculae) are passing through it, in the head its surface (the endost) is slightly irregular. Within the compact bony wall of the head some strips of collagen connective tissue are found and the superficial layer of its most proximal part consists of hyalin cartilage. This cartilage thus forms a dome shaped cartilage cupule at the proximal pole of the os priapi. It immediately adjoins the osseous tissue.

---

[1]) Acta. N. Morph. 2, 236, 1939.

The general bone lamellae being fairly distinct, in the cross sections especially the shaft shows a pattern like that of the annual rings in trees.

The solid body of the glans penis consists of fibrillar cartilage containing a dense complex of collagen fibres. It is surrounded by a distinct layer of connective tissue.

TABLE I.

Castrated young male rats, treated with testosterone.

Number of rats	Animal weight (extreme values)		Treatment	
	beginning	end	substance and dosage	during
4	99—132 g	121—169 g	300 γ testosterone-propionate	7 days
4	89—128 g	158—188 g	300 „ „	14 „
4	94—118 g	164—192 g	300 „ „	21 „

TABLE II.

Castrated and hypophysectomized young male rats, treated with testosterone and/or growthhormone. Castration 4 weeks before, treatment 1 week after hypophysectomy.

Number of rats	Animal weight (extreme values)		Treatment	
	beginning	end	substance and dosage	during
3	100—140 g	101—147 g	300 γ testost. prop.	28 days
3	87—131 g	108—138 g	10 U. growthhormone	28 „
5	101—132 g	138—154 g	test. prop. + growthhormone	28 „
4	89—126 g	86—122 g	olive oil[1]) and NaOH[2]) 0.01 N	28 „

Structure of the penis skeleton in the castrates.

In the cross sections the os priapi and the glans body appear as to be much smaller than normal: the diameter of the head is about 50 % and that of the shaft about 70 % of those diameters in normal animals of the same age. Only some of the sections being mounted, no information about the longitudinal dimensions is available. The difference between the degrees of hypoplasia in head and shaft are due to the fact that the diameter of the medullar cavity is more below the standard than the thickness of the bony wall. The internal (endostal) surface of the headwall is less irregular (more flattened) than normal.

The glans body of a castrate does not contain any cartilage cell: it thus exclusively consists of dense collective tissue.

Hypophysectomised castrates.

In the castrates, the hypophyseal gland of which has been removed, the hypoplasia of the penis skeleton is more marked yet. Especially the compact wall is very thin. Moreover its finer structure is somewhat irregular as if some shrinking has occurred. In the head the cartilage cupule is absent.

Influence of control injections.

As a control experiment, oil was injected in some of the animals. They did not show another microscopical picture than those without oil injections.

---

[1]) Solvent of testosterone propionate.

[2]) Solvent of growthhormone.

The influence of testosterone injected in castrates, the hypophyseal gland of which was not removed.

When testosterone is injected repeatedly into a castrate, the hypophyseal gland of which was not removed, the os priapi and the glans body are found to be larger than they would have been without these injections. After one week the diameter of the head is already twice as large as in an animal spayed at the same age and sacrificed after the same interval. During the following weeks this effect continues but decreasing in strength: the enlargement of the shaft and of the cartilage cupule stop after two weeks of treatment and in the 3d and 4th week the enlargements of the head and of the glans body are small.

During the first week the enlargement of the head is caused exclusively by a development of cartilage; at the end of that week the osseous tissue and the medullary cavity show the same conditions as in an untreated castrate, but the external surface of the bone is fully surrounded by a layer of new formed hyaline cartilage 2—3 times as thick as the original bone wall.

Then connective tissue, probably originating from the strips inside the bony wall, penetrate into the inner parts of the new formed cartilage layer. Very soon this connective tissue ossificates, so that after 2 weeks of treatment a thick layer of new formed bone is found all around the old bone tissue. The young bone is arranged in irregular rods; between them fields of connective tissue and of hyaline cartilage are scattered about. The bony rods tend to a radial direction and they grow towards the periffery. Their periferal parts tend to a mutal contact and at some spots they already reach the surface of the head.

After these two weeks the medullary cavity of the head is slightly enlarged: the endostal resorption of the original bone has begun at some spots, causing distinct irregularities of the internal (endostal) surface of the bony wall.

During the 3d and 4th week these processes continue, the growth of cartilage tissue at a rapidly decreasing rate and the ossification at a slowly decreasing rate while the endostal resorption at first increases in intensity and then decreases also. After 4 weeks of treatment the medullary cavity of the head is approximately as large as in the normal animals of the same age, the wall is somewhat thicker. Within that wall the cartilage tissue is building up an abnormally large proximal cupule with offshoots along the lateral surfaces. Within the bone tissue abnormally large and numeral fields of connective tissue are present, especially in the lateral parts of the head wall.

The periost of the head of the os priapi hypertrophies as well, especially during the 3d week.

The shaft of the priapis bone answers the testosterone injections by periostal growth of the bone. During the 3d week it reaches the thickness of a normal non-castrated animal of the same size. A distinct "annual ring" indicates the circumference of the original bone: obviously the shaft did not grow during the period (5 weeks) between the castration and the administration of testosterone. In the shaft the width of the medullary cavity does not alter during the testosterone treatment: here no endostal resorption occurs.

The glans body not only grows during the testosterone treatment — it reaches normal dimensions in 2 weeks — it shows a histological differentiation as well: in a castrate it being built up by connective tissue exclusively, cartilage cells appear in it as soon as testosterone is injected; the number of these cells increases during all the 4 weeks of the experiments and after these 4 weeks they are rather more than less numerous than normally.

Influence of testosterone upon castrates, the hypophyseal gland of which was removed.

After 4 weeks of treatment with testosterone injections, the hypophysectomized castrates show a microscopical picture that closely resembles that which may be expected in non-hypophysectomized castrates after $1\frac{1}{2}$ week of testosterone injections.

The external dimensions of the penis skeleton are between those of the latter with 1 and with 2 weeks treatment, the medullary cavity still has the extension as in the untreated

castrate of the same age. The strips of connective tissue, entering the bone from the endost, are somewhat hypertrophied. In the central parts of the new formed periferal cartilage layer some small bone rods have developed (in the non-hypophysectomized castrates this cartilage after 1 week of treatment does not yet contain any of such osseous rods and after 2 weeks many of them).

In many of these animals the growth of the head is asymmetrical. In the parts where the growth is less no cartilage is found; in stead of it between the periost and the original bone a thin layer of new formed bone can be found, which in all probability has been built by periostal ossification.

The shaft reveals the same picture as in a non-hypophysectomized castrate after 2 weeks of testosterone application.

The dimensions of the solid body in the glans, in contrary, correspond to those of the same stages in castrates possessing a hypophyseal gland: after 4 weeks treatment the number of cartilage cells is very large, even larger perhaps than in the non-hypophysectomized castrates with 4 weeks testosterone injection, and these cells themselves are larger.

Influence of Growth Hormone upon Hypophysectomized Castrates.

The administration of growth hormone for 4 weeks caused no more changes in the castrate than the development of a cartilage cupule in the wall of the head of the os priapi in 2 of 3 animals.

Influence of combined administration of Testosterone and Growth Hormone in the Hypophysectomized Castrate.

In the hypophysectomized castrate the combined injection of testosterone and growth hormone for 4 weeks produced a picture that practically corresponds with that in the non-hypophysectomized castrate after 2 weeks testosterone administration: the head is larger than after testosterone alone; the original medullar cavity is still recognizable, here and there the wall is arroded and considerable, rather irregularly out-lined processes are present. The major part of the head's wall consists of bone rods with much collagen connective tissue between them; the outer layer still consists of cartilage tissue. The size as well as the ossification of the cartilage and the endostal resorption of the original bone are approximatively at the same stage as in a non-hypophysectomized castrate after 2 weeks testosterone administration and distinctly further developed than in hypophysectomized, spayed recipients after 4 weeks exclusively testosterone.

After the combined administration of growth hormons and testosterone the shaft of the os priapi and the solid body in the glans of the hypophysectomized castrate show almost the same picture as after the application of testosterone alone for the same period.

Discussion.

The reaction of the penis skeleton upon the different injections described above has two aspects: a growth of the skeleton and structural changes.

The growth of the os priapi is due to an activity of the periost: in the head the periost builds cartilage, in the shaft it builds bone. The new formed cartilage has a much larger volume than the bone, formed in the same time. In consequence the head grows much more than the shaft.

Soon after the cartilage has developed, its ossification begins: from the strips of connective tissue lying within the osseous tissue of the head streams of connective tissue penetrate into the central parts of the cartilage and some days later the larger part of this new built connective tissue transists into bone. The young bone rods tend to a radial arrangement, they grow towards the perifery and there they grow towards each other. About a week after the beginning of the ossification the endost starts to resorb the central parts of the bony wall, thereby enlarging the medullary cavity.

In the shaft, where no ossification of cartilage occurs, the endostal bone resorption is absent.

When the growth stimulus is very week some parts of the periost in the head form bone in stead of cartilage. At these spots the growth is less than in the adjacent parts building cartilage.

The growth of the glans body is due to a hypertrophy of the connective tissue. Meanwhile cartilage cells develop within that tissue.

From the foregoing description it is obvious that in these reactions testosterone is the major stimulus (at least in the given dosages) in stimulating growth and producing structural changes and that the pituitary component acts mainly by *accelerating* these processes.

It is very probable, that this component may be identified with growth hormone:

1. The reappearance of the cartilagenous capsula on the head of the os priapi in the hypophysectomized castrate, receiving exclusively growth hormone.

2. The combined administration of testosterone and growth hormone to the hypophysectomized castrate produced a picture, resembling that of the non-hypophysectomized recipient, treated with testosterone for 2 weeks and much further developed than that of the hypophysectomized castrate, that received solely testosterone for 4 weeks.

Our experiments do not answer the question wether or not it is possible, to obtain the same results with a *chronic* application of testosterone alone as with the administration of testosterone to the non-hypophysectomized recipient for 4 weeks.

Conclusion.

The os priapi and the solid body in the glans are smaller and of other architecture in the castrate than in the normal animal of the same age.

The administration of testosterone produces a practically complete repair of size and structure. The course of this can be studied in 4 phases.

The administration of testosterone induces the same process of augmentation and differentiation in hypophysectomized as in non-hypophysectomized castrates, but at a lower speed. The rapidity of this process increases, when growth hormone is given at the same time, so this substance is capable of partially replacing the pituitary gland.

The nature of the process is not changed by the concomitant administration of growth hormone, as far as can be deduced from the histological preparations.

In the untreated castrate hypophysectomy causes only slight further changes; the latter are mainly expressed in the disappearance of the cartilage capsula in the head of the os priapi. The administration of growth hormone caused the reappearance of this structure.