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ON THE SEXUAL CYCLE IN THE REINDEER MALE

By

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Cervus tarandus (*Linnéus,* 1766), Rangifer tarandus (*Gray,* 1843), the reindeer is a common animal of the northern hemisphere and arctic areas from the 52nd parallel in Europe, Asia and America. Reindeer breeds may be subdivided into barren ground and woodland types. Rangifer tarandus Scandinavicus (Typicus) inhabits a large part of Norway, Sweden, Finland and extends into Russia and Siberia. Since the systematic relationships of the genus Rangifer are not known, it seems best to be conservative about the nomenclature of different breeds (*Lydekker,* 1898).

We know that breeding seasons occur but we are still ignorant of the precise combination of factors that initiate the sexual rhythms. There is an internal rhythm of reproduction which depends primarily upon the alternation of periods of rest and activity. In correlation with this rhythm hormones are periodically elaborated by the gonads and act upon the secondary organs and sexual characters.

Environmental factors act through the nervous system and hypothalamus upon the anterior pituitary and thence upon the testes or ovaries. In the male mammals the hypophysial and gonadal level of activity tend to rise and fall together. In the female the matter is complicated by the occurrence of pregnancy, and in polyoestrous species by the repetition of short dioestrus cycles (*Amoroso* and *Marshall,* 1960).

It is accepted that monoestrous wild living animals should possess the primary normal endocrine cycle, elucidation of which

should be helpful in understanding the sexual disorders occurring in domesticated animals.

The reindeer male was chosen for this investigation as being a phylogenetically old, monoestrous, half-domesticated ruminant of suitable size, possessing deciduous antlers, which undergo changes parallel with the sexual cycle.

It was decided to follow the sexual cycle, spermatogenesis, antler growth and urinary excretion of the Z.chr. in reindeer males kept under natural conditions, and in captivity as close as possible to natural conditions.

MATERIAL

The reindeer stags were kept in a large (2.5 acre) open paddock in a pine forest away from roads of communication, and 59°30′-north and 18°0′-east. One stag was studied separately for two years, and two other stags together for one and a half year. Two stags were studied for the next one year. No reindeer females were present in the area for at least five kilometres.

The feed consisted of green grass, hay, reindeer Cladonia and concentrates. Samples from animals kept under natural conditions were obtained from Lapland and Härjedalen areas during the years 1958 to 1962.

METHODS

Morning urine was collected for 5 days weekly throughout the year. A rubber apron with an enclosed plastic bag was put on the animal for about one hour. In this period an adequate urine sample was usually obtained. Samples from northern areas were obtained as required.

The urine was analysed using the Zimmermann m-dinitrobenzene colour reaction (Zimmermann modification, 1945). Approximately 1400 urine samples were analysed during this period of investigation.

Twenty one pairs of testicles were obtained from reindeer stags kept under natural conditions in the northern areas and killed at various times during the period of the study.

RESULTS

1. Testicles.

In the winter period, January to March, the testicles are small (about 15 g.) and in the scrotal position. Interstitial tissue seems

to be diminished and typical Leydig cells are rare. The tubular epithelium is reduced to one layer of cells on the basement membrane, and among these a few spermatogonia cells can be distinguished. Rarely a single primary spermatocyte is seen. No further cell differentiation is observed (Fig. 1).

In April the tubular epithelium is more organized. The number of the primary spermatocytes is increased and in solitary tubules active spermatogenesis is observed (Figs. 2, 3, 4). Sperm cells are found in the cytoplasmic network. Organisation of the germinal epithelium and the presence of ripening sperm cells in April indicate that initiation of spermatogenesis is correlated with initiation of antler growth which occurs at this time. Regeneration of the germinative epithelium is very slow during the summer. This is comparable with regeneration of the epithelium after warmth induced aspermia in the bovine (*Lagerlöf*, 1934, *Meschaks*, 1953). After scrotal insulation solitary spermatozoa appear very soon, but for complete regeneration several months are required.

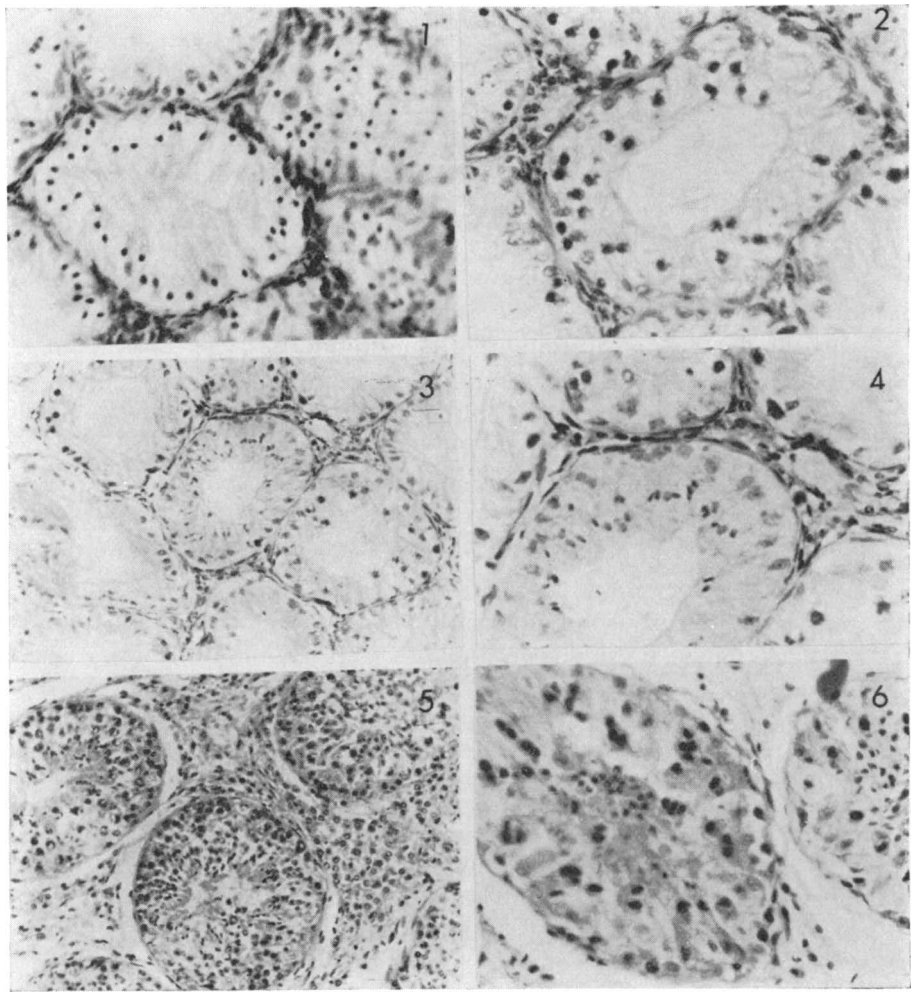
Testicular sections obtained in the beginning of August reveal a well organised tubular epithelium and abundant interstitial tissue with Leydig cells. In this period of regeneration signs of regression may be manifest, besides constructive changes. Sloughing of cells and pyknotic nuclei are observed.

At the time of shedding the velvet spermatogenesis is in progress and pyknotic nuclei rare. Testicular sections obtained in September (2 to 23) show that regeneration of the germinal epithelium is complete. Testicles are about 50 g.

The rutting season is essentially finished in the last days of October, but may continue until mid November. Testicular sections obtained November 23rd reveal a state of acute regression of the germinal epithelium. Some tubuli show active spermatogenesis, some sloughing of the germinal epithelium, pyknotic nuclei and polynuclear giant cells. The regression process is a rapid one and is accomplished by about Christmas time (Fig. 6).

2. The antler cycle.

The first antlers in reindeer calves develop during the first summer and are cast off in winter. These antlers are straight pin like formations four to six inches long and usually non-ramified. The first antlers develop long before sexual maturity. In the following years of life higher and ramified forms develop.



Figs. 1—6. Microphotographs. Seasonal changes in testicular tissue of reindeer. Haematoxylin-eosin.

1. January. Tubular regression to one layer of cells on the basement membrane, among which a few spermatogonia cells. (200 ×)
2, 3 and 4. April. Solitary tubuli may show spermatogenesis. (180 and 200 ×)
5. September. Full spermatogenesis. (200 ×)
6. November. Tubular regression, sloughing of the germinative epithelium, pyknotic cell nuclei. (200 ×)

Considerable variations in form and size occur individually. The antlers are deciduous i. e. they are cast off every year and new antlers develop at approximately the same time.

The data on the antler cycle were compiled from twenty breeding districts. Latitude, environmental conditions, climatic variations and the age of animals produce variations.

Initiation of the antler growth.

The first buds appear usually in March or April, occasionally in beginning of May, as soft black swellings vertical to the frontal bone. When of a hens egg size the formation is inclined at about 45°. From the distal end lateral and frontal branches are developed, and from the proximal end the large corona is formed. Initiation of the antler growth coincides with the beginning of regeneration of the testicular germinal epithelium.

Shedding of the velvet.

During the summer the antlers develop to their full size and harden. Shedding of the velvet occurs between August 10th and September 10th. The covering at the time of shedding seems to be in the best condition. The antlers are rubbed against trees and bushes, drops of blood may run over the face, and the shedding is finished within a few hours. Denuded antlers are partly stained with blood. The blood stained parts, when dry, become red-brown, the non-stained parts gray.

Soon after the shedding of the velvet the stags become aware of their weapons and become aggressive, even men can be attacked. This stage is associated with rapid development of the testicular tubular epithelium and active spermatogenesis. The testicles are enlarged.

Loss of the antlers.

Fully developed antlers persist only during the rutting time and are used for defeating rivals. After the rutting time they are cast off, which, however, occurs with a great variation of about five months — from November to May of the next year. Old well fed stags cast off their antlers earlier than young and undernourished individuals. Detailed observations are presented in the Table 1.

Table 1.
The antler cycle in experimental animals 1958—1961.

Stags no.	Age years	Initiation of growth	Shedding the velvet	Cast off
I.	2	—	—	5. 4. 1958
	3	29. 4. 1958	28. 8. 1958	1. 3. 1959
	4	9. 3. 1959	20. 8. 1959	5. 12. 1959
	5	29. 3. 1960	18. 8. 1960	23. 12. 1960
	6	13. 3. 1961	13. 8. 1961	15. 12. 1961
II.	3	2. 5. 1960	1. 9. 1960	11. 4. 1961
III.	3	5. 5. 1960	5. 9. 1960	22. 4. 1961
	4	29. 3. 1961	27. 8. 1961	15. 2. 1962

Experimental animals showed their antler cycle within the same time range as animals under natural conditions.

The tendency is that young animals keep their antlers in winter, older animals cast them off earlier. With increasing age the antler cycle begins earlier, particularly the stage of shedding the velvet and the casting off process.

3. Rutting period.

Oestrus in the female reindeer begins, with certain variations dependent on the latitude and climate, from September 20th to October 5th, and lasts for about two weeks under natural conditions.

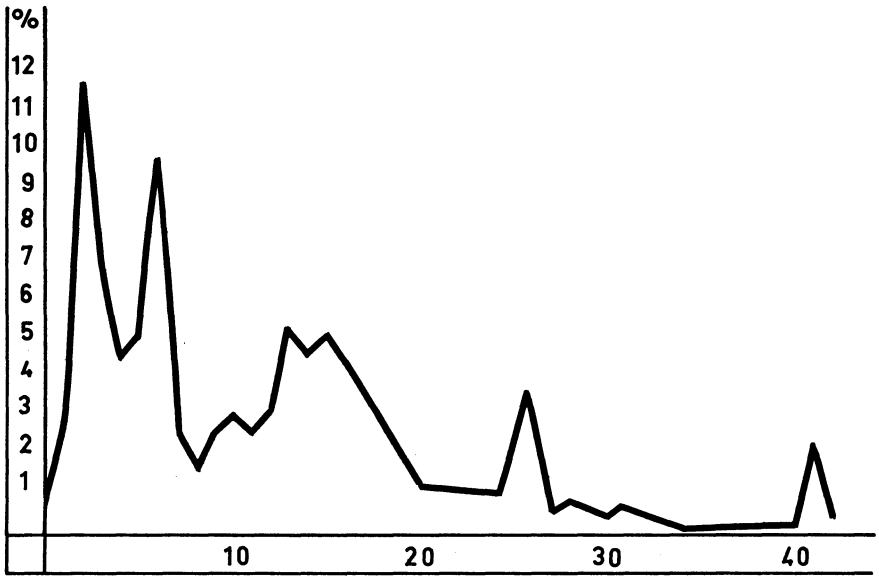
The rutting period can be characterised by normally developed spermatogenesis which under natural conditions is established about the middle of September and lasts until the middle of November i. e. for two months. In this manner the rutting period is guaranteed to cover the female oestrus.

Even during the rutting time the stags make no use of their voice. Snorting is a sign of aggression. The hair on the neck is erected and the muscles contracted but no real swelling of the neck could be observed. Protrusion of the non-erected penis occurs physiologically. The testes are enlarged. A peculiar musk like smell characterises the stags during the rutting time. The intake of feed is restricted in this time, and the stags loose much condition.

Spontaneous occurrence of the "male oestrus" thus becomes evident in animals kept separately for many years. All symptoms of rut appeared with great regularity in the absence of females.

4. Urinary excretion of the Zimmermann chromogen.

Separation of crude urine extracts from reindeer using the column and paper chromatography methods showed the presence of many constituents active in the Zimmermann reaction. Orientating analyses are made by *Plantin*. Individual oxo-steroids are not isolated (Fig. 7).



F i g. 7. Urine extract from reindeer. Column chromatography Al_2O_3.

Excretion of the Z.chr. in urine of reindeer stags was studied in three animals for seven periods and about 1400 urine samples were analysed. With regard to changes in the Z.chr. concentrations the calendar year can be subdivided into three periods:

The winter period lasting from Christmas to the beginning of March is characterized by low Z.chr. concentrations of about 12 to 15 mg./litre.

The spring-summer period, March to August, the Z.chr. concentrations are about 20 to 23 mg./litre. This excretion level continues until August with a short term increase during the initiation of antler growth and one higher increase during the change of the coat.

The autumn period begins in August with the shedding of the velvet. At this time the Z.chr. concentrations rise to 30—45 mg./

litre and remain at a high level until Christmas time, when they suddenly decrease to the winter level. With this period of high concentrations occur full spermatogenesis, rutting time and the regression of the germinal epithelium. It was observed also that illness may influence the excretion level.

DISCUSSION

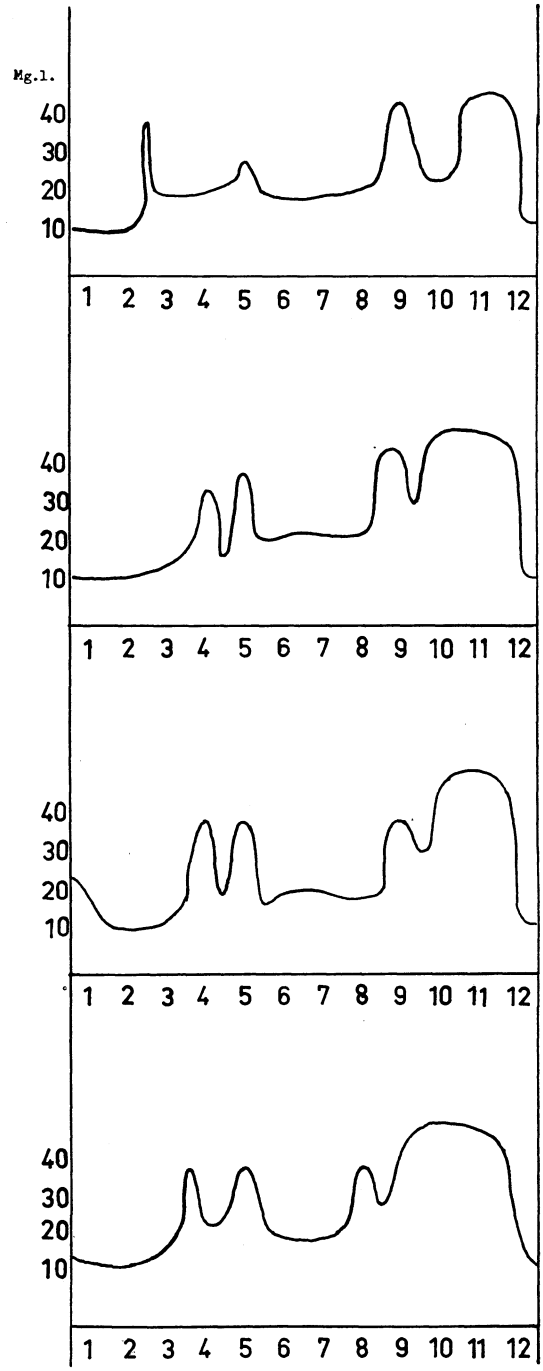
Testicular size in reindeer males shows considerable variations during the year. Small testicles with absent spermatogenesis in winter, and large testicles with active spermatogenesis during the rutting period in autumn occur physiologically from year to year. Thus the testicular regression and regeneration should be controlled by factors regulating the sexual cycle i. e. the central nervous system and the sexual hormones.

Analysing the spermatic vein blood it has been found that testes of domestic animals produce comparatively large amounts of testosterone, androstenedione, 17-hydroxyprogesterone (*Lindner,* 1961) and dehydroepiandrosterone (*Neher and Wettstein,* 1960). Testicles of one old bull produced 11 milligrams of testosterone in one hour.

From bovine adrenals a number of steroidal hormones have been isolated by *Reichstein* (1936). Many steroidal metabolites of the C_{19}-type were isolated from urine of animals (*Neher* and *Wettstein,* 1960). Difficulties of obtaining pure steroids from urine extracts are well known. Observations, however, show that purification procedures are associated with loss of the substances in proportion to the degree of purification, particularly in bovine (*Unger, Rosenfeld* and *Dorfman,* 1961, *Binder,* 1959, and *Stock,* 1957).

It was expected that changes in concentrations of the Z.chr. in urine of reindeer would correspond with the hormonal state during different seasons of the sexual cycle, even if crude urine extracts were used. This actually was the case. Concentrations of the Z.chr. in winter were low 12—15 mg./litre), increased early in the spring (20—23 mg./litre) and were high (30 to 45 mg./litre) in autumn. These variations occurred regularly in all three stags observed for seven seasons, therefore they are regarded as an expression of the sexual cycle in reindeer males (Figs. 8, 9, 10, 11).

Variations in the concentration of the Z.chr. appear also



Excretion of the Zimmermann chromogen in urine of reindeer males.

Fig. 8. A separately kept reindeer stag.

Figs. 9, 10 and 11. Three stags kept together in a paddock.

during the initiation of antler growth, change of the coat and disease.

In reindeer both sexes develop antlers while in most deer species the females are polled or both sexes antlerless (Musk deer). Since Aristoteles the antler cycle and effects of castration in deer species have been known. Castrated deer males (Odocoileus Virginianus and Capreolus capreolus) develop abnormal antlers. Administration of "oestrogen" (*Blauel,* 1935, 1936) or testosterone (*Wislocki,* 1947) induce normal antler growth, and even antler growth in polled females.

Tandler and *Grosz* (1913) report that castrated males and spayed females of reindeer develop normal antlers. Castrated males may develop even higher and heavier antlers as compared with intact animals. From the information obtained from native reindeer breeders, still ancient methods of castration are in use, and none of these methods is associated with removal of the testes. "Weakly castrated" stags behave like entire animals, shed the velvet at the usual time and cast off their antlers after the rutting season. These animals usually develop higher and heavier antlers. Naturally very many degrees of testicular damage may occur, therefore the results vary. "Hard castrated" animals usually develop smaller antlers, the castrates are indolent during the rutting period and never shed their velvet. The velvet is desiccated and the antlers are cast off in their coverings next spring. New antlers start to grow in April and May.

In cross sections the antlers or normal intact males show a large substantia ossea and small substantia spongiosa. Hard castrated stags develop antlers with small substantia ossea and a large spongiosa.

These observations indicate the role of the testicular hormone in antler development in reindeer males.

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SUMMARY

Reindeer are monoestrous half-domesticated ruminants in which cyclic changes occur in spermatogenesis, antler growth, behaviour, and urinary excretion of the Zimmerman chromogen (Z.chr.). These cyclic changes occur with great regularity even if the stags are kept in the absence of females for many years.

The winter period, January and February, is characterized by small testicles and low Z.chr. concentrations in the urine (12 to 15 mg./litre).

The spring-summer period, March to July, is a period of reactivation, initiation of spermatogenesis, antler growth and change of coat. Concentrations of the Z.chr. in this period are about 20 to 23 mg./litre. The antlers usually are cast off.

The autumn period, August to December, begins with shedding of the velvet, then full spermatogenesis and the rutting period which is followed by postoestral regression of the germinative epithelium. The Z.chr. concentrations are high in this period (30 to 45 mg./litre).

Indications are that the Z.chr. level in the urine of male reindeer is an expression of the endocrine state, marking the sexual cycle and metabolic events.

ZUSAMMENFASSUNG

Über den Sexualzyklus bei männlichen Renntieren.

Das Renntier ist ein monoestraler halbdomestizierter Wiederkäuer, der zyklische Veränderungen in der Spermatogenese, Hornwachstum, Sexualbenehmen und in der Exkretion der Zimmermann Chromogenen im Harn aufweist. Diese zyklische Veränderungen kommen regelmässig vor, auch wenn die männlichen Tiere für mehrere Jahre von den weiblichen getrennt sind.

Kleine Hoden und niedrige Z.Chr. Konzentrationen (12—15 Mg/L.) sind für das Winterperiod, Januar-Februar, typisch. Die meisten Bullen verlieren zu dieser Zeit ihr Geweih.

Im Vorjahr und Sommer ist eine Periode der Reaktivierung, wo die Spermatogenese anfängt, das Geweih wächst, und die Winterhaare gewechselt werden. Die Z.Chr. Konzentration zu dieser Zeit ist etwa 20—23 Mg/L.

August-Dezember ist eine Periode der Geweihreifung, Hodenvergrösserung, Rut und postoestralen Regression des germinativen Epithels. Die Z.Chr. Konzentration ist etwa 30—45 Mg/L.

Die Konzentration der Z.Chr. ist ein Zeichen der hormonalen Aktivität während der Sexualcyklus und Stoffwexelvorgänge.

SAMMANFATTNING

Undersökningar över sexualcykeln hos ren tjurar.

Renen är en monoestral, halvtam idisslare, som visar cykliska förändringar i spermatogenesen, horntillväxten, det sexuella beteendet och i exkretionen av Zimmermannkromogenerna i urinen. Dessa cykliska förändringar förssiggår mycket regelbundet, även om handjuren hålles borta från hondjuren under flera år.

Små testiklar och låg Z.kr. koncentration i urinen (12—15 mg/l) är typiskt för vinterperioden, januari-februari. De flesta tjurarna fälla hornen under denna tid.

Vår- och sommarperioden, mars-juli, är en period av reaktivering, då spermatogenesen begynner, hornen börjar växa och vinterhåren fälls. Koncentrationen av Z.kr. under denna period är omkring 20—23 mg/l.

Höstperioden, augusti-december, inledes med att djuren fejar sina horn, spermatogenesen utvecklas, och därefter följer parningstiden. Denna åtföljes av post-oestral regression av det germinativa epiteliet.

Z.kr. koncentrationen i urinen hos tjurar är ett yttryck för de endokrina funktionerna under sexualcykeln och den allmänna ämnesomsättningen.

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