Cyclical Changes in the Distribution of the Testis Lipids of a Seasonal Mammal (*Talpa europaea*)

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With two plates (figs. 1 and 2)

Summary

In the mole, *Talpa europaea*, there occurs a sequence of interstitial events comparable with that of seasonal birds, reptiles, and some fishes. There is a periodic accumulation and discharge of cholesterol and lipids in the Leydig cytoplasm. This cycle differs from its avian counterpart in that whereas in birds (and some fishes) the Leydig cells rapidly discharge their sudanophil contents before mating, the interstitium of the mole becomes exhausted much more slowly. Another difference is that the Leydig cells of the mole do not appear to become exhausted and replaced *en masse* as in birds after spermatogenesis. The seasonal growth and regression of the mammalian accessory sexual organs can be correlated with the increase and decrease of secretory activity of the interstitial cells (as shown by their lipid cycle). In the seminiferous tubules of the mole there is no lipid cycle corresponding to that observed in sub-mammalian vertebrates.

Introduction

As the seasonal spermatogenesis and sexual behaviour heightens in birds (Marshall, 1949, 1954), in at least one reptile (Marshall and Woolf, 1957), and in a fish (Lofts and Marshall, 1957), there is a progressive diminution of the Schultz-positive (i.e. cholesterol-containing) lipids in the endocrine cells of the testis, followed by their post-nuptial regeneration in preparation for the next sexual season. Further, after the shedding of spermatozoa, the residual contents of the seminiferous tubules undergo a massive steatogenesis accompanied by the appearance of quantities of cholesterol. Lofts and Marshall (1959) have shown that this material probably contains progestins.

Although such tubular lipids arise in mammals after hypophysectomy (Coombs and Marshall, 1956), their natural presence has not been shown in any mammal, although small amounts of lipid material arise (lipophanerosis) during normal mammalian spermatogenesis. Few, however, have been studied to this end (Wislocki, 1949).

The purpose of the present study was to examine the interstitium and seminiferous tubules of an emphatically seasonal mammal in order to discover whether cyclical events occur, comparable with those reported in sub-mammalian vertebrates.

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Material and Methods

It was considered that if tubule steatogenesis does occur in the Mammalia, the mole (*Talpa europaea*, Linn.), which undergoes great variation in testis size, might exhibit the phenomenon.

The seasonal changes in the genitalia of this animal have been studied in detail by several authors (Krasa, 1918; Courrier, 1927; Schwarz, 1928; Tandler and Grosz, 1912, 1913; and others). The majority of these workers, however, used traditional wax-embedding techniques which result in the dissolution of cytoplasmic and other lipids. Thus, many aspects of the testis cycle escaped notice.

The material for the present study consisted of 117 adult moles collected over a period of two years from January 1957 to October 1958 inclusive. Whenever possible the testes were dissected out and preserved within an hour after death, but in a number of cases (42) fixation was unavoidably delayed for periods of up to 24 h. The histological condition of the latter, however, did not appear to be much affected by post-mortem changes. After measurement of the whole gonad, testicular material was subjected to the following uniform procedures:

(1) One sliced organ was immersed in Bouin's fluid and subsequently embedded in wax. This material was sectioned at 6 μ and stained with iron haematoxylin and orange G for routine examination of spermatogenetic stages. (2) The remaining testis was fixed in formaldehyde-calcium solution, embedded in gelatine, sectioned at 8 μ on the freezing microtome, coloured with Sudan black, and stained with haemalum for the investigation of tubule and interstitial lipids. (3) Sections were subjected to the Schultz test for the detection of cholesterol. All measurements were made on the material embedded in wax.

Results

Seasonal variations in size

The breeding season is short and is accompanied by great changes in the reproductive anatomy of both sexes, including a pronounced testicular expansion similar to that of birds and fishes. Table 1 shows that during March and April the gonads are at their maximum size of 17×10 mm, and subsequently undergo a very rapid regression to a minimum of $5{\cdot}5 \times 4{\cdot}5$ in July. There now ensues a period of inactivity during which the testes remain small. In January they again become active and expand rapidly throughout February.

Seasonal histological changes

January. The testis tunic is about 50 μ thick and the regressed tubules measure 80 to 90 μ in diameter. The tubules contain only spermatogonia (fig. 1, A) and are without sudanophil material or cholesterol. The interstitium is extensive and consists of large, rounded, Leydig cells measuring 12×10 μ with a nucleus 2 μ in diameter. The majority of the Leydig cells are completely free from cytoplasmic lipids, but some isolated cases do occur

where the Leydig cytoplasm contains a few minute lipid droplets which are negative to the Schultz test.

TABLE I

Seasonal variation in testis size of Talpa europaea

Month	*No. examined*	*Testis size* (mm)
January .	6	10×5·9
February .	11	13×7·9
March .	10	17×10
April . .	8	14·5×7
May . .	11	8×5
June . .	9	6·5×4
July . .	7	5·5×4·5
August .	12	8×6·5
September .	10	8×6
October .	8	8×6
November .	14	8×5
December .	11	8×5

February. Tubule expansion has begun and the diameter is now 140 μ. The stretched testis coat is reduced to a width of 40 μ. Spermatogenesis has begun and the tubules now contain primary and secondary spermatocytes. In the interstitium an increasing number of Leydig cells are accumulating Schultz-positive lipids in their cytoplasm (fig. 1, B). The tubules are lipid-free.

March. The tubules have continued to expand owing to the ripening products within, and are now 250 μ in diameter. The tunic is reduced to a thickness of 30 μ. Bunched spermatozoa now occur. Within the lumen of some of the tubules there is a sparse scattering of minute lipid droplets (similar to those described by Wislocki (1949) in the testes of autumn-breeding deer). This is the normal vertebrate process of lipophanerosis which occurs during the transformation of spermatids into spermatozoa and has nothing in common with the massive post-nuptual tubule steatogenesis that occurs in birds and fishes. The expanded tubules have dispersed the interstitium. The Leydig cells are more lipoidal and Schultz-positive but comparatively few are strongly sudanophil (fig. 1, C).

April. The histological condition is little changed from that of the previous month. The tunic width is 30 μ, and the tubules are 230 μ in diameter and contain spermatozoa. There has been a considerable increase in the number of lipoidal Leydig cells (fig. 1, D).

May. Specimens collected during this month fell into two well-defined groups.

(1) Six specimens had testes with a histological appearance similar to that of the animals caught in the preceding month. The tubules were 220 μ in diameter and contained masses of spermatozoa (figs. 2, A, B). The interstitial Leydig cells were extensively lipoidal and many contained dense masses of sudanophil material and cholesterol.

(2) Five moles had discharged their spermatozoa and, as a consequence, tubule diameter had become reduced to a mere 50 μ. Spermatogonia only were now present and the tubule lumina were devoid of sudanophil material and cholesterol. The interstitium still consisted of large, densely lipoidal and Schultz-positive Leydig cells (fig. 2, C).

June. The testes are still small with a shrivelled tunic, 20 μ thick. The seminiferous tubules are unchanged, being 48 μ in diameter and containing only a few spermatogonia. The interstitial Leydig cells are less heavily charged with Schultz-positive lipids.

July. A proliferation of fibroblasts is laying down a new testis tunic within the old weakened coat (fig. 2, D). This double tunic is 85 μ wide and surrounds tubules 60 μ in diameter. No post-nuptial lipids have arisen in the tubules. The only germinal elements now are a peripheral layer of spermatogonia which, in most tubules, is a single cell deep. The Leydig cytoplasm still retains a little lipid and cholesterol but is much less sudanophil than in the June specimens.

August. The tunic is now 50 μ wide and most of the old coat has disintegrated. The tubules remain unchanged at 60 μ diameter with no germinal cells in advance of spermatogonia. The Leydig cells have now lost most of their lipid and cholesterol.

September to December. The histological appearance of the testis remains constant. The organ is ensheathed in a single new tunic 50 μ thick and there is an extensive interstitium surrounding tubules 70 to 90 μ in diameter. These remain inactive with only a peripheral ring of spermatogonia. The Leydig cells are denuded of sudanophil material and are negative to the Schultz test.

Discussion

The interstitial cycle

The interstitial cycle of the mole has been studied by several workers (Tandler and Grosz, 1912, 1913; Courrier, 1927; Schwarz, 1928), who have advanced a number of conflicting viewpoints. According to Courrier (1927) these cells remain fairly constant throughout the year, not varying conspicuously in number or size. Tandler and Grosz (1912, 1913), on the other hand, reported that they vary inversely with the activity of the adjacent

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Fig. 1 (plate). A, testis in early January, showing regressed inactive seminiferous tubules containing only a peripheral ring of spermatogonia. The interstitium is extensive and contains large Leydig cells in a non-secretory condition. Bouin fixation; 6 μ paraffin-wax section stained with iron haematoxylin and orange G.

B, testis in February. Spermatogenesis has begun and the expanded tubules contain primary spermatocytes as well as spermatogonia. Leydig cells are starting to accumulate Schultz-positive lipid droplets. Formaldehyde-calcium fixation, 8 μ gelatine section coloured with Sudan black and haemalum.

C, testis in March. Tubules contain spermatids, and tubule-expansion has constricted the interstitial tissue. Technique as in B.

D, testis in April. Tubules are at the height of spermatogenesis. The interstitial Leydig cells are now densely lipoidal and Schultz-positive. Technique as in B.

seminiferous tubules, being smallest and least numerous during the period of maximum spermatogenesis and largest and most numerous during the anoestrous period. Further, Courrier also states that the interstitial cells contained rich cytoplasmic lipid all the year round. In my material this did not appear to occur.

The interstitium undergoes a well-defined cycle involving a gradual accumulation of lipid and cholesterol in the cytoplasm of the Leydig cells. This begins in January with the appearance of a few small sudanophil droplets in a few Leydig cells, and continues throughout the following months until nearly all the interstitium becomes involved and all Leydig cells contain dense Schultz-positive lipids. Maximum lipid content coincides with breeding activity and sperm-shedding in April and May. There now follows a gradual disappearance of sudanophil material from the interstitium, so that by late July to early August it is completely absent, and remains so until January.

This interstitial cycle of the mole is basically similar to that of birds in that it involves a seasonal accumulation and discharge of lipids and cholesterol. But whereas in birds and fishes the Leydig cells gradually discharge the sudanophil contents before mating, the interstitium of the mole becomes exhausted much more slowly and the testis still contains much Leydig lipid and cholesterol a month after sperm-shedding.

The testes of birds and the pike are almost never without lipid and cholesterol. During the immediate post-nuptial period, when a new generation of Leydig cells has arisen but has not yet accumulated sudanophil droplets, the neighbouring seminiferous elements have metamorphosed and contain large quantities of lipid and cholesterol in the tubule lumina (Marshall, 1949; Lofts and Marshall, 1957). While this is gradually clearing from the tubules through the winter months, sudanophil material begins to accumulate in the cytoplasm of the Leydig cells. The interstitium of the mole, on the other hand, has a period from September to December when it is devoid of lipid and cholesterol. Another point of difference is that in the mole the Leydig cells do not appear to become exhausted *en masse* and to be replaced by a new generation of juvenile cells, as occurs in birds after reproduction. The interstitium at all times appears to consist of rather large cells which remain more or less constant, apart from their lipid content, throughout the year. They appear somewhat larger and more numerous during the autumn and winter months because, during oestrous, the great expansion of the seminiferous tubules compresses

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FIG. 2 (plate). A, testis in April, showing tubules containing all stages of spermatogenesis including free spermatozoa. Technique as in fig. 1, A.

B, gelatine section of April testis, showing the fine lipid particles that are produced during lipophanerosis. Technique as in fig. 1, B.

C, a 'spent' testis in the last week of May, showing great decrease in size of the tubules. These have discharged their spermatozoa and are without any sudanophil material. The interstitium still contains densely lipoidal and Schultz-positive Leydig cells. Technique as in fig. 1, B.

D, part of the testis tunic in July. A proliferation of new fibroblasts is forming a new testis tunic within the old, weakened coat of the regressed gonad. Technique as in fig. 1, A.

the interstitial tissue into tight wedges occupying the small interstices where several tubules meet, the cells between adjacent tissues becoming squeezed and compressed under the pressure of the ripening germinal elements within the lumina. It was probably this phenomenon that led Tandler and Grosz (1912, 1913) and Lecaillon (1909) to state that the interstitial cells showed their most marked development in the anoestrous period and decreased in number as spermatogenesis advanced. In fact, the present study has shown that interstitial activity is at its *highest* during the latter phase and this is supported by the parallel cycles of the accessory reproductive organs. Thus in March the vasa efferentia, epididymis, vasa deferentia, prostate, and Cowper's glands are greatly hypertrophied, and their epithelial elements are in a secretory condition (see Eckstein and Zuckerman, 1956). During the post-nuptial period the regression of these structures is rapid and closely follows the disappearance of Schultz-positive lipids from the cytoplasm of the Leydig cell cytoplasm, being complete by early July.

Tubule lipids

The only lipids observed in the tubules were minor amounts resulting from lipophanerosis, which is characteristic of vertebrate spermatogenesis. Very few mammals have been studied during every month of the year by techniques that would retain post-nuptial tubule lipids if they were in fact present. However, such do not occur in man (Montagna, 1952), vole (Marshall and Wilkinson, 1956), deer (Wislocki, 1949), nor, as has been demonstrated in the present study, in the European mole. These species represent a fairly wide selection of eutherian mammals. Monotremes and marsupials have not been studied in this connexion.

In the mole, and probably other seasonal mammals as well, part of the basic seasonal rhythm undoubtedly occurs. Thus, the interstitium undergoes a rhythmical lipid accumulation and depletion. However, at no time do the seminiferous elements undergo a metamorphosis involving the production of large masses of lipoidal material and cholesterol in the tubule lumina such as occur in some fishes (Lofts and Marshall, 1957), reptiles (Marshall and Woolf, 1957), and birds (Marshall, 1954).

Laboratory rats and mice, of course, show spermatogenetically active tubules all the year round. The interstitium is lipoidal and Schultz-positive throughout life. The seminiferous tubules at no season produce large quantities of sudanophil material or cholesterol, nor do prolonged and massive daily injections of prolactin cause tubular steatogenesis in mammals such as occurs in passerine birds (Lofts and Marshall, 1956). Yet hypophysectomy in rats leads to tubular steatogenesis (with the accompanying production of cholesterol), essentially similar to that which occurs naturally (and also after hypophysectomy) in birds. The interstitial cell tissue, however, does not regenerate as it does after removal of the anterior pituitary in domestic cockerels (Coombs and Marshall, 1956) and pigeons (Lofts and Marshall, 1958). Lacy and Rotblat (1958) have demonstrated that irradiation of rat testis also brings

about a massive accumulation of Schultz-positive lipids in the tubule-lumina.

It is of phylogenetic interest that the testes of the Chelonia, Crocodilia, and Lacertilia contain Leydig cells with seasonally variable lipid content, and seminiferous tubules that undergo a post-spermatogenetic steatogenesis (Marshall and Woolf, 1957). In view of the common reptilian ancestry of birds and mammals, it seems possible that mammals too once possessed this tubular mechanism, which is still functional in seasonal birds. In mammals it has subsequently been lost as a naturally recurring phenomenon, though the mole, and no doubt many other seasonal mammals, still retain an interstitial cycle.

The testis tunic

As in birds, reptiles, and fish, a new testis tunic is manufactured seasonally by a resurgence of fibroblasts within the old wall. This phenomenon is probably common to most animals whose gonads undergo great seasonal enlargement. Such expansion, and the subsequent rapid contraction after spermatozoa are shed, weakens the testis coat, which then becomes replaced by a new tunic beneath, before the old one finally disrupts and disappears.

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