THE TESTIS OF THE AFRICAN ELEPHANT (*LOXODONTA AFRICANA*)

I. HISTOLOGICAL FEATURES

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Summary. Testis tissues from thirty-five elephants collected in Uganda, East Africa, were studied. The histology of the elephant testis was found to correspond closely to generalized descriptions for other mammals. The morphology of each cell type is described and illustrated.

The cellular composition (in percentages) of each tissue sample was obtained by using the Chalkley technique. The relative abundance of intratesticular components in mature elephant testes are compared with like data for the human and rat.

Nine phases were developed to express various cell associations of both immature and mature seminiferous epithelium. Analyses revealed that older animals frequently possessed a quantity of immature tubules. This phenomenon may reflect pubertal effects and/or cyclic influences. The phase values of the elephant are compared with those of several other mammals.

INTRODUCTION

The reproductive characteristics of elephants are poorly understood. Several authors: Krumbiegel (1943), Hill (1953), Deraniyagala (1955) and Frade (1955) provide works of a relatively comprehensive nature. Only one study (Perry, 1953) offers detailed information on elephant reproduction. Most of the last work involves the female reproductive cycle with only a few comments pertaining to the male. Other materials on elephant reproduction are fragmentary. Mojsisovics (1879), Oudemans (1892) and Schulte (1937) describe the anatomy of the male reproductive system, but offer few comments at the microscopic level.

With the exception of the works mentioned above, present knowledge of the elephant rests upon a body of information which is largely of a non-scientific, questionable, often anecdotal nature. There is a paucity of well substantiated data on the various phases of elephant biology. Thus, it is hoped that the findings reported in this paper (the first of two treatments dealing with certain morphological and quantitative features of the testes) will enhance and extend the limited information available on reproduction in this animal.

METHODS

Testicular tissues were obtained from fifty-eight elephants collected from July 1958 to May 1959 in Bunyoro and Toro districts of Uganda (Text-fig. 1). Tissue samples were excised from the mid-portions of the testes and fixed in formalin. The massiveness of the animals, plus the difficulty of the terrain,

TEXT-FIG. 1. Map of the collection area (numbers show the approximate location where each specimen was killed).

prevented prompt removal and fixation of the material in some cases. Of the fifty-eight animals, the tissues of thirty-five are represented in this paper. This selection reflects histological samples of high quality. The rejected tissues were autolyzed to a degree that precluded objective histological examination.

Following the removal of the desired tissues, each elephant was measured and weighed; and, its teeth removed and saved for later study. The measurements and weights were related to the teeth (Johnson & Buss, 1965) in such a way that the age of each specimen was approximated.

The testis samples were shipped to Washington State University where they were processed in a Technicon. Preparations were cut at $7\ \mu$ and stained in Heidenhain's haematoxylin and eosin.

The technique developed by Chalkley (1943) was employed to secure quantitative estimates of the cellular composition of each testis sample. This technique involves a series of random fields under oil immersion from which are recorded the elements appearing at the tips of four pointers set into the ocular lens of a microscope. In order to determine an appropriate sample size, slides representing five animals were sampled at 100, 150, 200, 250 and 300 oil immersion fields with the results calculated at each of the above levels. On the basis of the calculations, a sample size of 150 fields/slide was selected. Counts beyond this level produced only slight variations. To substantiate further the soundness of the sample size, five slides (each representing a different animal) were analysed twice as a test of reproducibility. In all cases the variation between replications was insignificant.

In addition to the Chalkley technique, the phase analysis procedure was employed to obtain quantitative information about the testes. This analysis is based upon a series of phases each of which shows a certain cellular association of the seminiferous epithelium, and which in aggregate represents one spermatogenic cycle (Leblond & Clermont, 1952). A series of nine phases was developed which deviated somewhat from the generally used procedure. A description of these phases will be given subsequently.

To make the analysis, testicular preparations were scanned under low power and each transversely sectioned tubule was assigned a phase value. After an adequate number of tubules was sampled, an evaluation of the relative frequency of each phase was obtained. It was found that 200 tubules/individual was a sufficient sample size. Replications were readily made, and sampling beyond this level produced no significant difference in the end result. The sample size selected was comparable to that used by Roosen-Runge & Giesel (1950).

RESULTS AND DISCUSSION

CELLULAR COMPONENTS

This section describes the major features of the cells comprising the seminiferous epithelium and interstitium. Some of the terms below (chromatin flakes and granules, for example) are meant in a purely descriptive way, and do not refer to any functional characteristics of the chromosomal system.

Spermatogonia

Two types of spermatogonia (similar to those described for the laboratory rat by Leblond & Clermont, 1952; and Roosen-Runge, 1955) were found in the elephant testis. The two types (designated A and B) can be described as follows:

(1) Type A spermatogonia (Pl. 1, Figs. 1 and 2) are relatively large cells (14 to $15\ \mu$ in diameter). The nucleus shows little affinity for stain, and typically contains a single large karyosome (some nuclei show one to several smaller karyosomes). Throughout the nucleus, fine chromatin flakes are characteristic.

Nuclear shape varies from round to oval, and the nuclei are bounded by a fairly distinct membrane. Cytoplasm is evident, and is usually distributed elliptically with the long axis of the cytoplasmic mass parallel to the basement membrane.

Spermatogonia in immature testes (Pl. 3, Figs. 7 and 8) are similar to those described above. They show prominent nucleoli as do the Type A cells, but differ in that they appear to contain less cytoplasm and to have more irregularly shaped nuclei.

(2) Type B spermatogonia (Pl. 1, Figs. 1 and 2) are readily separable from Type A cells, since they display a characteristic nuclear pattern and are smaller in size (8 to 9 μ in diameter). The chromatin material of the B cell is abundant and coarse, and tends to adhere to the nuclear membrane resulting in a 'beaded' appearance of the membrane. This sort of membrane morphology has been referred to in the literature as 'chromatin crust' (Roosen-Runge & Giesel, 1950; Ortavant, 1959).

Primary spermatocytes

Newly differentiated primary spermatocytes in the elephant are similar to those described for domestic mammals (Ortavant, 1959) and for the rat (Roosen-Runge & Giesel, 1950). The latter authors refer to these cells as 'pre-spermatocytes'. The early spermatocytes resemble the Type B spermatogonia from which they originate. However, they lose the 'chromatin crust' of the latter, and also their nuclei tend to stain more densely (Pl. 1, Figs. 2 and 3).

By the time the early spermatocytes become recognizably different from their B cell precursors, they have increased in size to a diameter of 9 to 10 μ . Cytoplasmic and nuclear growth continues, and ultimately these cells form the conspicuous mature primary spermatocytes. Apparently the initial darkly stained nucleus of the newly formed spermatocyte results from chromosomal contraction. Such a condition is well known in the domestic bull (Ortavant, 1959). When the cells reach mature size (14 to 15 μ in diameter), the chromatin material forms a characteristic net-like pattern within the nucleus (Pl. 1, Fig. 3 and Pl. 2, Fig. 4).

Newly formed spermatocytes were not recognizable in the testes of immature animals. The only definite spermatocytes seen (Pl. 3, Fig. 8) were morphologically similar to mature primary cells.

Secondary spermatocytes

These cells are relatively difficult to find in mammalian testes since equational divisions are completed rapidly. Secondary spermatocytes (10 to 11 μ in diameter) were occasionally seen in the elephant material, and were distinctive in appearance (Pl. 2, Fig. 4).

The nucleus is intermediate in size relative to that of the primary spermatocyte and spermatid, and cytoplasm is extensive and prominent. The nuclear membrane appears thick, probably as a result of adjacent chromatin material. Flakes and filaments of chromatin are conspicuous within the typical nucleus. The distribution of the chromatin causes secondary spermatocyte nuclei to

PLATE 1

Figs. 1–3. Photomicrographs of mature seminiferous epithelium (approximately $\times 900$).

(Facing p. 14)

PLATE 2

FIGS. 4-6. Photomicrographs of mature seminiferous epithelium (approximately $\times 900$).

PLATE 3

FIG. 7. A photomicrograph of immature testicular tissue from a 2-year-old elephant (approximately $\times 400$).

FIG. 8. A photomicrograph of immature testicular tissue from a 4½-year-old elephant (approximately $\times 400$).

FIG. 9. Photomicrograph showing a group of Leydig cells in which the cytoplasm is vacuolated (approximately $\times 1900$).

resemble strongly those of Type B spermatogonia. Primary and secondary spermatocytes in division display prominent spindle fibres (Pl. 2, Fig. 4).

Spermatids and spermatozoa

Early spermatids are essentially smaller replicas (8 to 8.5 μ in diameter) of secondary spermatocytes. Like their precursors, they display a conspicuous cytoplasmic fraction. Spermatid nuclei do not stain as darkly as those of secondary spermatocytes, but are surrounded by a distinct membrane (Pl. 1, Fig. 1).

The overall morphological changes which accompany spermiogenesis were noted. One of the first observable nuclear modifications is an increased affinity for stain. Concomitant to this change there is a sloughing of cytoplasm (spermiogenetic debris). Large quantities of such material occupy tubules where spermiogenesis is occurring (Pl. 2, Fig. 5). Further changes involve the gradual lengthening and flattening of the nucleus. Ultimately, the spermatid nucleus assumes a flattened ovoid shape, and the flagellum is produced. In the spermatozoon thus formed, the head-piece (nucleus) stains lightly (Pl. 2, Fig. 6), and a thin relatively indistinct mid-piece is present. Measurements of fifty spermatozoa indicate that the head-piece is about 6 μ in length, and approximately 2.5 to 3 μ in width; the mid-piece is about 8.6 μ in length and only slightly thicker than the flagellum. Measurements of flagellar length are difficult to obtain from sectioned tissues, and the longest intact flagellum recorded was 47.0 μ . Thus, the minimum length of an entire spermatozoon would be 61.6 μ .

The head-piece morphology discussed above conforms to that described for the Indian elephant (*Elephas maximus*) by Schulte (1937). Size measurements, however, are different. Schulte describes the 'body' as being 8.3 μ in length with the 'tail' equal to five times this value (or 41.5 μ). Apparently the term 'body' refers to the head-piece, and the mid-piece was not delimited. Thus the heads of Indian elephant spermatozoa are probably larger than those of the African elephant. Possibly, however, the overall length of African elephant spermatozoa exceeds that of the Indian elephant, since Schulte has probably combined both the mid-piece and tail region in his 41.5 μ value.

Sertoli cells

The cytoplasm of these cells is obscure, but the nuclei with their irregular membranes and prominent nucleoli are conspicuous (Pl. 2, Fig. 6).

Immature testes (before the establishment of active spermatogenesis) possess tubules containing large numbers of Sertoli cells arranged in palisade-like fashion (Pl. 3, Figs. 7 and 8). These early cells are smaller than the mature form, and do not display prominent nucleoli. Their nuclei, however, are characteristically irregular and their cytoplasmic boundaries are not readily discernable. Ortavant (1959) calls cells of this type 'supporting cells', and considers them to be Sertoli cell precursors. He shows in certain domestic animals that these cells proliferate actively for a time, then cease, with the progeny gradually transforming into the mature Sertoli cells.

Measurements of entire Sertoli cells were impossible, due to the obscure outline of the cytoplasm. The nuclei of mature cells range from 12 to 15 μ in length and 6 to 9 μ in width.

Leydig cells

Leydig cells are abundant and prominent in the interstitium (Pl. 3, Fig. 9). Their nuclei vary in staining intensity, and the cytoplasmic portions of each cell are generally definable. The cells are usually round in shape, with some being oval to elongate. Cellular shape seems to be influenced by forces within the interstitial 'islands'. Cells located centrally are usually round in outline, while those peripherally located seem more compacted and frequently are elongate. Leydig cell diameters varied from 8 to 14 μ . This size variation may be associated with relative secretory activity.

TABLE 1
COMPOSITION OF MATURE ELEPHANT TESTES

Specimen No.	Approximate age (years)	Ratio of testis weight (g) to body weight (kg)†	Mean tubule diameter (μ)	Percentage relative abundance*						
				Extra-tubular area	Intra-tubular area	Spermato- gonia	Spermato- cytes	Spermato- id-spermatozoa	Sertoli cells	Space
90	13	1.06	169	20.3	79.7	5.0	19.1	26.6	6.5	22.4
85	14	0.94	161	22.5	77.5	4.5	18.5	28.7	6.2	19.5
91	15	0.98	171	23.0	77.0	4.4	24.6	21.2	8.4	18.2
3	19	0.90	187	22.9	77.1	5.2	20.7	31.0	1.9	18.1
116	19	1.16	163	27.2	72.8	3.6	21.6	31.2	5.6	10.7
25	25	1.02	163	26.9	73.1	4.9	19.7	27.0	3.7	17.5
84	30	0.96	189	25.1	74.9	5.0	21.5	25.7	3.5	19.0
Mean		1.00	172	24.0	76.0	4.6	20.8	27.3	5.1	17.9

* The extratubular area represents 'hits' (by the pointer tips) within the interstitial materials or 'hits' on the connective tissue sheath which bounds each seminiferous tubule. The space designation indicates 'hits' on cytoplasm of unknown affinity, in open spaces within the seminiferous epithelium, or within the lumen of a tubule. A given cell type was recorded whenever a pointer rested on a nucleus or within cytoplasm that could be associated readily with a nearby nucleus.

† Ratio = $\frac{\text{Weight of testes}}{\text{Body weight (kg)}}$

QUANTITATIVE FEATURES OF THE TESTES

The Chalkley analysis

The thirty-five elephants represented in this paper ranged in approximate age from 1 to 30 years. As would be expected, varying degrees of testicular maturity were reflected by varied compositional data as revealed by the Chalkley technique. Some features of this variation will be described in a later paper dealing with the pattern of intratesticular development.

For present purposes, we shall restrict our discussion of the Chalkley data to a group of seven mature animals in which testicular development (as expressed in testis- to body-weight ratios) was comparable. In this way, a quantitative picture of the composition of mature testicular tissue can be given and compared with similar information for other species. A compilation of the data for these specimens is shown in Table 1.

The Chalkley technique has not yet been used widely enough to provide extensive mammalian data comparable to that for the elephant. However, the

elephant findings can be contrasted (Table 2) with similar data for the human and rat (Roosen-Runge, 1956). There are obvious differences among these species relative to testicular composition. For example, the spermatogonia and spermatid-spermatozoa fractions of the elephant appear to be intermediate to those of man and rat, while the spermatocyte and Sertoli cell fractions are higher and lower, respectively. Since there is no information available on the rate of spermatogenesis in the elephant, the functional significance of these differences cannot be evaluated.

The phase analysis

The idea of phases or periods characterizing the cycle of spermatogenesis dates back to the work of Ebner (1888) and Regaud (1901). These investigators showed that the spermatogenic epithelium possesses an inherent orderly cyclesuch that a series of readily identifiable cell associations characterizes the spermatogenic process. They furthermore identified twelve 'periods' or cell associations typical of the cycle in the rat. Subsequent to their preliminary work, the phase

TABLE 2
A COMPARISON OF THE TESTICULAR COMPOSITIONS* OF
THE HUMAN, THE RAT AND THE AFRICAN ELEPHANT

<i>Components</i>	<i>Human</i>	<i>Rat</i>	<i>Elephant</i>
Extratubular area	34.2	12.1	24.0
Intratubular area	65.8	87.9	76.0
Spermatogonia	7.8	1.7	4.6
Spermatocytes	14.4	14.7	20.8
Spermatid-spermatozoa	9.1	41.1	27.3
Sertoli cells	17.4	8.4	5.1
Space	16.4	20.6	17.9

* Figures represent the percentage of each item relative to the entire testicular mass.

analysis method has been used widely in spermatogenic studies. Two principal approaches have been developed. One (proposed by Leblond & Clermont, 1952) uses spermiogenesis (especially the development of the acrosomic system) as the basis around which the various phases are delimited. The other, and more widely used method (Roosen-Runge & Giesel, 1950; Ortavant, 1959; Amann, 1962), involves a series of phases which consider the entire cellular association within each tubule sampled. The various phases are based upon meiotic divisions, variations in the shape of spermatid nuclei and the release of spermatozoa from the tubule. This latter approach is more readily employed than the former, and hence a variation of it was applied to the elephant material.

Previous investigators have used the phase method as a means to characterize the spermatogenic cycle ('the series of changes occurring in a given area of the seminiferous epithelium between two successive appearances of the same cellular association', Ortavant, 1959) of the mature testis. The present study, however, involves a different situation in that the materials represent variably aged animals. We decided that the best approach would be to establish a group of phases

which could be applied to the entire study series, such that all specimens could be placed on a comparable basis regardless of relative maturity. With this in mind, a series of nine phases was developed, some of which are patterned after those of Roosen-Runge & Giesel (1950).

The criteria used to delimit each respective phase are as follows:

Phase 1. Immature tubule, with a sparse germ cell population and with no tubular lumen.

Phase 2. Immature tubule, first indications of spermatocyte formation, Sertoli cells generally common.

Phase 3. Primary spermatocytes present, secondary spermatocytes may be infrequently present in very small numbers, and low numbers of spermatids.

Phase 4. As outlined for phase 3, except spermatozoa are sparsely present in the tubule.

Phase 5. No spermatozoa present, abundant spermatids which are either undergoing elongation in the central area of the tubule or becoming associated with Sertoli cells.

Phase 6. Spermatid bundles well organized, maturation divisions among primary and secondary spermatocytes are occurring.

Phase 7. Spermatozoa bundles are moving centrally, toward the lumen.

Phase 8. Spermatozoa have completed their central movement and are all located within the lumen of the tubule.

Phase 9. Spermatozoa absent from the tubule, abundant spermatids which are not yet elongating.

Phases 1 to 4 apply particularly well to the young animal with immature testes, but also occur (especially phases 3 and 4) in the testes of older animals. Phases 5 to 9 are strictly characteristic of mature tubules, and hence represent a simplified version of the more elaborate phase series used by other workers. With respect to the phases advanced by Roosen-Runge & Giesel (1950), the present phase 9 corresponds to their phase 1, phase 5 corresponds to their phases 2 and 3, phase 6 corresponds to their phases 4 and 5, phase 7 corresponds to their phases 6 and 7, and phase 8 corresponds to their phase 8. This correspondence allowed comparison of the elephant with other species in which the Roosen-Runge and Giesel phases were used.

As shown in Table 3, phases 1 and 2 are almost entirely restricted to young elephants possessing immature testes, with only two occurrences of phase 2 tubules among the older specimens. Phases 3 and 4, however, are common in the older animals. There were only four specimens (Nos. 23, 3, 116 and 25) where the tubules were completely restricted to the mature phases (5 to 9). Possibly, the presence of immature tubules in the older animals represents an effect of puberty. Segal & Nelson (1959) note that in human testes some tubules may remain immature for several years following the completion of pubertal development. It also seems possible that this phenomenon reflects a subtle cyclic flux of the testicular epithelium. Perhaps the constancy of the elephant's tropical environment has produced a cyclicity in spermatogenic function similar to that seen in the domestic bull (Mercier & Salisbury, 1946; Clegg & Ganong, 1959; Kihlstrom, 1962) or the domestic sheep (Maqsood, 1951; Terrill, 1962).

In order to compare the phase analysis of the elephant with comparable data from the literature, a compilation was made using the data for testes which contained only mature tubules (phases 5 to 9). The compilation represents only three animals (Nos. 3, 116 and 25), since No. 23 seems at variance

TABLE 3
THE RELATIVE FREQUENCY (IN PERCENTAGE) OF SPERMATOGENIC PHASES
IN ELEPHANT TESTES

Specimen No.	Approximate age (years)	Spermatogenic phases									
		1	2	3	4	Percentage below 5	5	6	7	8	9
60	1	100.0	—	—	—	100.0	—	—	—	—	—
74	2	100.0	—	—	—	100.0	—	—	—	—	—
96	3½	100.0	—	—	—	100.0	—	—	—	—	—
55	4½	59.5	40.5	—	—	100.0	—	—	—	—	—
94	6	66.5	33.5	—	—	100.0	—	—	—	—	—
98	6	63.5	36.5	—	—	100.0	—	—	—	—	—
34	6	20.0	80.0	—	—	100.0	—	—	—	—	—
52	7	43.0	57.0	—	—	100.0	—	—	—	—	—
95	7	35.5	51.0	8.5	5.0	100.0	—	—	—	—	—
61	8	11.0	39.5	43.5	6.0	100.0	—	—	—	—	—
64	8	2.5	32.0	45.0	20.5	100.0	—	—	—	—	—
87	9	—	—	5.0	31.5	36.5	20.5	9.5	18.5	3.5	11.5
1	10	—	—	22.5	31.5	54.0	9.0	6.5	26.5	0.0	4.0
8	11	—	—	9.5	12.5	22.0	6.5	25.0	26.5	14.0	6.0
76	11	—	—	6.5	10.5	17.0	15.5	17.5	21.5	13.5	15.0
10	12	—	—	6.5	13.5	20.0	14.5	17.5	21.0	12.5	14.5
19	12	—	—	25.5	20.5	46.0	15.0	12.0	19.0	3.0	5.0
26	12	—	—	—	2.0	2.0	21.5	20.5	29.5	6.5	20.0
35	13	—	6.5	14.0	34.0	54.5	11.0	13.5	9.5	3.5	8.0
79	13	—	—	4.0	13.5	17.5	15.5	19.5	24.5	9.0	14.0
90	13	—	—	9.5	8.0	17.5	13.0	19.0	32.0	3.5	15.0
85	14	—	—	11.5	7.5	19.0	13.5	19.5	34.5	4.5	9.0
91	15	—	—	11.0	5.0	16.0	14.5	20.0	30.0	5.5	14.0
78	16	—	19.0	38.5	38.5	96.0	1.5	0.5	2.0	—	—
23	17	—	—	—	—	0.0	28.0	8.0	28.5	8.5	27.0
89	17	—	—	4.5	9.5	14.0	18.5	20.0	31.0	4.5	12.0
124	17	—	—	3.0	3.5	6.5	12.5	22.5	30.5	4.5	23.5
42	18	—	—	2.5	13.0	15.5	11.5	25.0	17.5	13.0	17.5
3	19	—	—	—	—	0.0	23.5	21.0	19.0	16.5	20.0
116	19	—	—	—	—	0.0	19.0	22.5	24.5	14.5	19.5
86	19	—	—	5.5	4.5	10.0	12.5	22.5	25.5	11.5	18.0
119	20	—	—	3.5	7.5	11.0	22.5	16.0	23.5	10.0	17.0
121	22	—	—	6.0	5.5	11.5	14.0	15.5	28.5	10.5	20.0
25	25	—	—	—	—	0.0	24.5	17.5	19.0	15.5	23.5
84	30	—	—	8.5	5.5	14.0	17.0	20.5	28.5	4.5	15.5

with the other data (very low phase 6 value). The resultant percentage calculations indicate the following means: phase 5 = 22.3, phase 6 = 20.3, phase 7 = 20.8, phase 8 = 15.5, phase 9 = 21.0. Table 4 compares the elephant data with several other mammals all of which were analysed using the eight phases

advanced by Roosen-Runge & Giesel (1950). Table 4 illustrates a distinct shift in the proportion of the cycle comprising the last four stages. This fraction varies from 26% to 31% in domestic bulls to 72% in rats. The last four phases are stressed, since new spermatids appear in phase 5, remain in the tubule during the current cycle and are not released as spermatozoa until the end (phase 8) of the succeeding cycle. Thus, as Ortavant (1959) points out, the

TABLE 4
THE SPERMATOGENIC PHASES OF THE ELEPHANT COMPARED TO SIMILAR INFORMATION
FROM OTHER MAMMALS

Phases	Species*						
	Domestic bull	Domestic ram	Monkey	African elephant	Domestic boar	Laboratory mouse	Laboratory rat
1 to 3	59 to 63	51	49	43	30	23	23
4	8 to 12	10	7	10†	14	10	5
5 to 8	26 to 31	38	42	47	57	67	72

* All data (except for elephant) are from Amann (1962).

† The value for phase 4 unfortunately could not be readily determined from our data since the Roosen-Runge phase 4 was included in our phase 6. Hence, the value shown was estimated on the basis of the other species. The Roosen-Runge phase 4 is that phase involving the equational divisions. Such tubules were infrequent in the elephant material, and a 10% estimate seems reasonable.

relative frequency of spermiogenesis can be expressed as 126% for the bull or 172% for the rat. In the case of the elephant, the spermiogenesis frequency is about 147%. Amann (1962) notes that the physiological significance of these differences is obscure.

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