QUANTITATIVE FEATURES OF SPERMATOGENESIS IN THE MALLARD (*ANAS PLATYRHYNCHOS*)

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THE usual approach in studying the seasonal pattern of avian spermatogenesis is to utilize a series of stages, each representing a particular level of intratesticular development (Blanchard and Erickson, 1949; Johnston, 1956; Williamson, 1956; Johnson, 1961). Although this method has been a useful one, it rests upon a degree of inherent subjectivity and does not readily permit the detection of subtle differences. The technique developed by Chalkley (1943) furnishes a method by which these problems can largely be eliminated.

The purposes of this paper are to demonstrate the possibility of greater accuracy in characterizing the avian testicular cycle by using the Chalkley technique, and to describe in quantitative terms the testicular cycle of the Mallard (*Anas platyrhynchos*).

METHODS

The specimens were collected in southeastern Washington in 1958 and 1959. Testes were excised immediately upon death and fixed in AFA. Slides (cross sections) were made from the mid-portions of the left testis of each individual. The tissues were stained with Heidenhain's hematoxylin and counterstained in eosin.

The birds also furnished data for an earlier paper (Johnson, 1961). In the latter work, each preparation was subjectively evaluated and assigned a stage designation (from 1 to 7) descriptive of various levels in the testicular cycle. For this study, many of the same preparations have been re-evaluated with the Chalkley technique. This method involves a series of random oil immersion fields from which one records the elements appearing at the tips of four pointers set into the ocular lens of a microscope. With a sufficient number of sample fields one can obtain a quantitative evaluation of a piece of tissue. Roosen-Runge (1955, 1956), Barry (1962) and Kennelly and Foote (1964) also have used this technique for the study of testicular tissues.

The size of the sample necessary to ensure reliable quantification was determined by taking 100, 150, 200, 250, and 300 sample fields from each of five slides representing birds at different points in the reproductive cycle. The results were compared at each of the above sampling levels. Only slight changes were noted after 150 fields and fluctuations after 200 fields were almost non-existent. Therefore, the 200 field sample size was selected as an adequate representation of each preparation.

RESULTS

A summary of the data obtained is shown in Table 1. The various constituents recorded for each testis are relatively self-explanatory, but a few comments seem necessary. A given cell-type was recorded whenever the tip of a pointer rested on a nucleus or within cytoplasm that could be

associated with a nearby nucleus. The space designation indicates occurrences where the pointer was located either on cytoplasm of unknown affinity or in open spaces between the cells of the seminiferous epithelium. The lumen category denotes the centrally located space bounded by seminiferous epithelium. Basement membrane refers to the well organized connective tissue sheath which surrounds each seminiferous tubule.

The data in Table 1 were divided for each bird such that the extratubular components (interstitial connective tissue, Leydig cells, and basement membrane) were considered as a testicular fraction separate from the intratubular components (cells of the seminiferous epithelium, space, and lumen). The percentage composition of each of these two fractions was calculated independently of the other. Individual variation in the seminiferous epithelium or within the extratubular materials is better demonstrated by this separation.

Table 1 is based upon the definitions of stages used earlier for these tissues (Johnson, 1961: 352). Stages 1 through 6 represent the gradual development from immature to completely mature tissue. No data are shown for the period of regression (stage 7) since calculations representing tissue degeneration are illogical. The newly calculated percentages were first grouped according to the spermatogenic stage previously assigned to each individual. It became apparent that certain individuals did not compare favorably in terms of tissue constitution with other birds in the same stage. This was evidence of the inherent subjectivity involved in the

TABLE 1

MEAN COMPOSITION (IN PER CENT) OF MALLARD TESTES IN VARIOUS SPERMATOGENIC STAGES

Constituents	*Stages*[1]					
	1 (14)	*2 (5)*	*3 (6)*	*4 (2)*	*5 (3)*	*6 (13)*
	All components[2]					
Intratubular	68.7	78.7	81.6	83.4	82.9	87.5
Extratubular	31.3	21.3	18.4	16.6	17.1	12.5
	Intratubular components[2]					
Spermatogonia	5.4	13.8	28.0	21.6	18.1	10.5
Spermatocytes	0.6	12.6	27.4	38.7	36.7	30.8
Spermatids and sperm	0	0	0.3	11.0	20.3	40.1
Sertoli cells	66.2	54.7	25.0	12.5	9.2	5.2
Space	25.0	18.0	19.3	15.2	13.2	9.0
Lumen	2.9	0.8	0	0.8	2.4	4.2
	Extratubular components[2]					
Interstitial connective tissue	67.7	53.0	56.7	62.7	65.4	69.4
Leydig cells	8.5	13.1	9.7	5.0	4.6	2.5
Basement membrane	23.7	33.8	33.6	32.1	29.8	28.0

[1] Numbers in parentheses are the number of birds studied for each stage.

[2] Based on 100 per cent.

assignment of spermatogenic stages without objective analysis. Thus, some birds were shifted to adjacent stages wherein they more closely matched the other individuals. Approximately one-third of the specimens in stages 1 through 5 had to be re-assigned to the next lower or higher category. These alterations did not result in contradiction of earlier interpretations (Johnson, 1961). Excessive variation in testicular composition was not encountered among specimens originally assigned to stage 6 (full maturity). Thus, in this category, prior evaluations were substantiated by the Chalkley analysis.

DISCUSSION

The effects of subjectivity mentioned above indicate the desirability of establishing spermatogenic stage categories by objective means. The percentage composition approach seems to be the most logical method of achieving this end. With this technique, the placement of any individual in the overall scheme depends solely upon quantitative criteria. Admittedly, the procedure is relatively tedious and time-consuming. However, a practiced observer, aided by a person to record the data, can easily evaluate a preparation in less than an hour. In my opinion, the reduction of subjectivity by this additional expenditure of time is worthwhile. I do not mean to imply that the older method of testicular analysis has been unsatisfactory. The objective approach is preferable, however, in those cases where a more critical evaluation of avian spermatogenesis is desired.

The curves (Figure 1) depict the status of the seminiferous epithelium during spermatogenic stages 1 through 6. These curves are based upon the data in Table 1 and reflect the differences between stages more strikingly than do the numerical values in the table.

The cellular organization in stage 1 displays a relatively low occurrence of both spermatogonia and spermatocytes (5.4 and 0.6 per cent, respectively) with an emphasis upon Sertoli cells (66.2 per cent). Deviations from these mean values were interpreted as indications of either a more immature level of stage 1 development or activation toward the stage 2 level. Thus, it appears possible to recognize subtle differences in relative maturity among individuals assigned to the same spermatogenic stage.

Increased populations of spermatogonia and spermatocytes are well in evidence by stage 2. This increase continues to the stage 3 condition wherein development has proceeded to the first sparse occurrence of spermatids and sperm (0.3 per cent). The latter fraction increases rapidly to attain a value of 40.1 per cent by stage 6.

The high relative percentage of Sertoli cells in stage 1 with declining percentages during the recrudescence process, indicates a probable constancy in absolute numbers within a given testis. Similar findings have

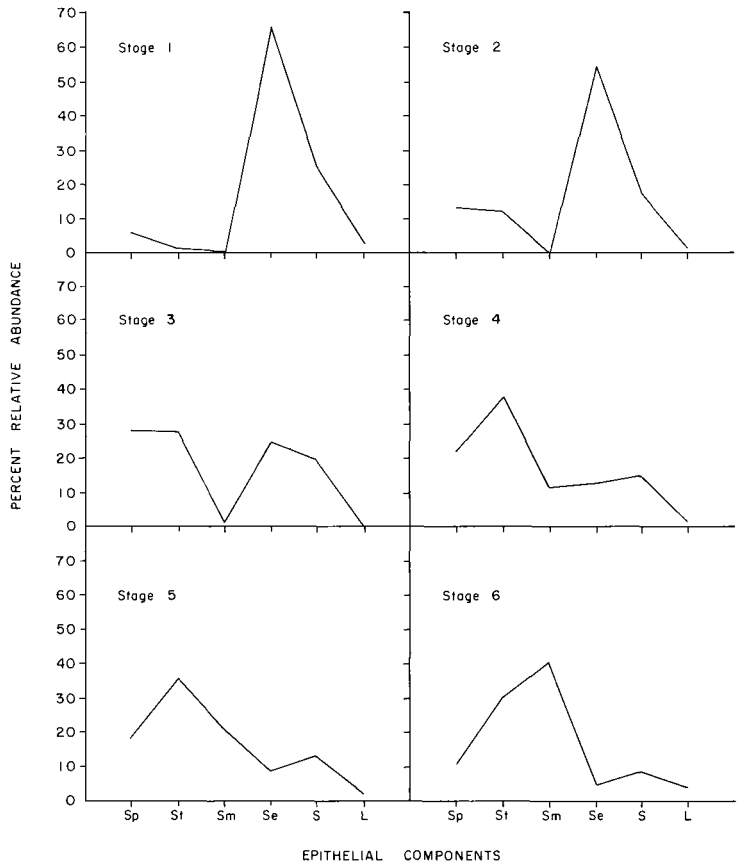

Figure 1. The relative abundance of the components of the seminiferous epithelium during spermatogenic stages 1 through 6 (Sp = spermatogonia, St = spermatocytes, Sm = spermatids and sperm, Se = Sertoli cells, S = space, L = lumen).

been reported in the laboratory rat (Roosen-Runge and Giesel, 1950: 3) and in domestic mammals (Ortavant, 1959: 35).

One can visualize the organizational changes occurring during the cycle from inactive to fully active testicular tissue by scanning Table 1 from left to right. If information were available as to the time spent in each stage of the cycle by average individuals a truer perspective of these changes could be presented. Tissue samples taken at intervals from a captive flock might yield information on this point. Actually, if the time element were known, the stage values would no longer be needed as reference points around which to organize the pattern of tissue change. It would then be possible to represent the testicular cycle as a continuum based upon a time scale.

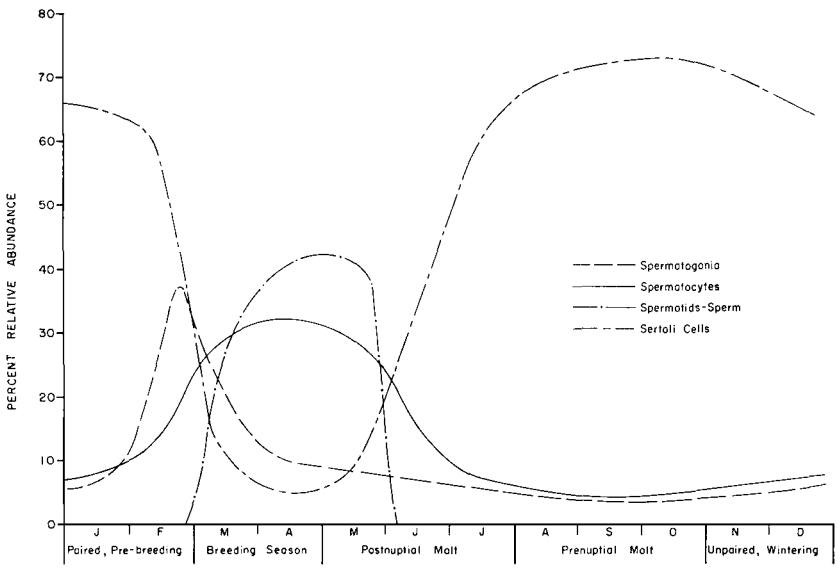

Figure 2. The status of the seminiferous epithelium during major periods of the annual cycle (see text for further explanation).

An attempt along these lines is represented in Figure 2. The graph shows the changing composition of the seminiferous epithelium during the annual cycle. The curves are based upon the testes of 25 adult birds. The subdivisions of the year represent major events within the Mallard population of the study area. The approximate duration of each of these subdivisions was revealed by general field observations and by observing the behavior of individuals immediately prior to collecting them. The changing sequence of intratesticular composition can be considered as an approximation of the testicular status in the population at various times. The curves can also be interpreted as a rough indication of the cycle in an individual drake. It should be noted, however, that the regression process (during the postnuptial molt period) is more rapid on an individual basis (Johnson, 1961: 358), than is indicated in Figure 2. Also, the relative duration of each of the subdivisions shown in the figure is more characteristic of the population than it is for individuals.

A notable feature of the interstitial tissue should be mentioned. As shown in Table 1, Leydig cell abundance is maximal during the first three spermatogenic stages, and then appears to decline during the remainder of the cycle. This phenomenon probably results from the two factors described by Marshall (1961: 188): first, it seems well established

that the expansion of seminiferous tubules leads to a dispersal of interstitial elements; and secondly, many Leydig cells appear to disintegrate in avian testes prior to the advent of full spermatogenesis. Without histochemical evidence one cannot relate the apparent changes in cell abundance with functional aspects. Further studies on the Mallard testis should provide more conclusive evidence as to the functional cycle of the Leydig cells.

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SUMMARY

The Chalkley technique represents a method through which the relative abundance of testicular components can be readily determined. This quantitative approach lessens the subjectivity associated with the assignment of spermatogenic stages by mere observation of a slide. The method was used to define more precisely the spermatogenic stages employed in earlier work on Mallard testes.

The changing organizational status of Mallard seminiferous epithelium is shown in conjunction with the major phases of the annual cycle. With adequate information, it would be possible to ignore the spermatogenic stage approach, and represent the testis cycle as a continuum wherein the relative abundance of each cell-type is considered.

Leydig cells appeared to be most abundant during early phases of the gonadal cycle.

LITERATURE CITED

BARRY, T. W. 1962. The effect of late seasons on Atlantic Brant reproduction. J. Wildl. Mgmt., **26**: 19–26.

BLANCHARD, B. D., AND M. M. ERICKSON. 1949. The cycle in the Gambel Sparrow. Univ. California Publs. Zoöl., **47**: 255–317.

CHALKLEY, H. W. 1943. Method for the quantitative morphologic analysis of tissues. J. Nat. Cancer Inst., **4**: 47–53.

JOHNSON, O. W. 1961. Reproductive cycle of the Mallard duck. Condor, **63**: 351–364.

JOHNSTON, D. W. 1956. The annual reproductive cycle of the California Gull. I. Criteria of age and the testis cycle. Condor, **58**: 134–162.

KENNELLY, J. J., AND R. H. FOOTE. 1964. Sampling boar testes to study spermatogenesis quantitatively and to predict sperm production. Anim. Sci., **23**: 160–167.

MARSHALL, A. J. 1961. Reproduction. Pp. 169–213 *in* Biology and comparative physiology of birds, vol. 2 (A. J. Marshall, ed.). New York, Academic Press.

ORTAVANT, R. 1959. Spermatogenesis and morphology of the spermatozoan. Pp. 1–

50 *in* Reproduction in domestic animals, vol. 2 (H. H. Cole and P. T. Cupps, eds.). New York, Academic Press.

ROOSEN-RUNGE, E. C. 1955. Quantitative studies on spermatogenesis in the albino rat. Anat. Rec., **123**: 385–398.

ROOSEN-RUNGE, E. C. 1956. Quantitative investigations on human testicular biopsies. Fert. and Steril., **7**: 251–261.

ROOSEN-RUNGE, E. C., AND L. O. GIESEL. 1950. Quantitative studies on spermatogenesis in the albino rat. Amer. J. Anat., **87**: 1–30.

WILLIAMSON, F. S. L. 1956. The molt and testis cycles of the Anna Hummingbird. Condor, **58**: 342–366.

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