Development of the Testis Tubule in the Fowl

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With three plates (figs. 1–3)

Summary

Three phases of tubule development can be distinguished in males of the Fayomi breed:

(i) the first 12 weeks, when only slight increase in tubule diameter and cell count occurs. During this period two stages in spermatogenesis are to be found.

(ii) from the 16th to the 24th week, when rapid increase in tubule diameter and cell count occurs, and all stages of spermatogenesis are present. Sexual maturity, judged by the appearance of the first spermatozoa in the seminiferous tubules, occurs at about 16 weeks and is associated with a rapid increase in the size of the testes. The increase in testis weight is due, however, to increase in length of the tubules rather than to increase in diameter.

(iii) from the 24th to the 52nd week, when only slight changes occur in microscopical appearance.

Introduction

In normal male fowls, four developmental stages of spermatogenetic activity have been observed (Hiatt and Fisher, 1947; Kumaran and Turner, 1949; Charny, Conston, and Meranze, 1952). The first occurs during the first 5 weeks, when the tubules are organized and gradually increase in diameter. The spermatogonia also multiply during this first period. In the second phase, primary spermatocytes begin to appear in the sixth week, and during the next 2 to 3 weeks the growth of the layer of primary spermatocytes takes precedence over further multiplication of the spermatogonial layer. In the third phase, secondary spermatocytes begin to appear at about 10 weeks. During the fourth stage, spermatids appear in the tubules of birds 12 weeks old. By the twentieth week, spermatids are usually present in all the seminiferous tubules. During this period, there is also marked growth in length of the tubules, and thus the capacity of the testes to produce spermatozoa is increased (Kumaran and Turner, 1949).

Sexual maturity is reached with the appearance of the first spermatozoa in the seminiferous tubules, at about 16 weeks. The age at which this occurs varies according to breed, locality, management, and nutrition. In pen-matings, however, a satisfactory level of fertility may not be reached until the cockerels are 26 weeks old (Hogue and Schnetzler, 1937; Parker, McKenzie, and Kempster, 1942).

The present study was designed to follow in detail the process of tubule development in males of the Fayomi breed.

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Material and Methods

Thirty-nine Fayomi males were killed at ages ranging from 4 to 52 weeks at intervals of 4 weeks. Up to the time of killing they received normal, balanced rations, and rearing and management were standard. Groups of these birds were weighed alive and then killed in groups of 3 at the following ages and on the following dates:

Age in weeks	*Date of killing*	*Age in weeks*	*Date of killing*
4	2 December	32	21 July
8	28 December	36	18 August
12	25 January	40	15 September
16	22 February	44	18 October
20	29 March	48	15 November
24	24 April	52	14 December
28	1 June		

The testes were dissected out and weighed immediately on a torsion balance. The average weight of the pair of testes per bird at each age (given in the Appendix, p. 405) was calculated by dividing the total weight of right and left testes from all three males at each age by three.

Samples were taken from the middle of each of the two testes of the three individuals in each age-group. The samples were fixed in Bouin's fluid, washed, dehydrated, cleared, embedded in paraffin wax, sectioned at 10 μ, stained with haematoxylin and eosin, and mounted in Canada balsam. Sections from each age-group were examined microscopically, and the different stages of spermatogenetic activity were noted. Microscopical examination was carried out with a projector microscope. The number of seminiferous tubules in each projector field was counted, and the number per square cm of section was calculated. The diameters of the seminiferous tubules were measured in microns. For this purpose tubules were usually chosen that were circular in cross-section; when oblique sections were measured, the diameters of the shorter and longer axes were averaged.

The number of seminiferous tubules was determined in 120 separate microscopical fields from 10 slides for each right and left testis of each of the 3 individuals in each age-group. The number of cells per tubule was also counted in one of the seminiferous tubules in each of these microscopic fields. To standardize the counts, tubules of circular section were chosen. Sections and microscopical fields in which shrinkage due to fixation was evident were avoided. Photographs of one of the sections in each age-group were taken with the projector objectives $\times 10$ and $\times 30$.

Fig. 1 (plate). Sections of testes. A, at 4 weeks, autopsied 2 Dec.
B, at 8 weeks, autopsied 28 Dec.
C, D, at 12 weeks, autopsied 25 Jan.

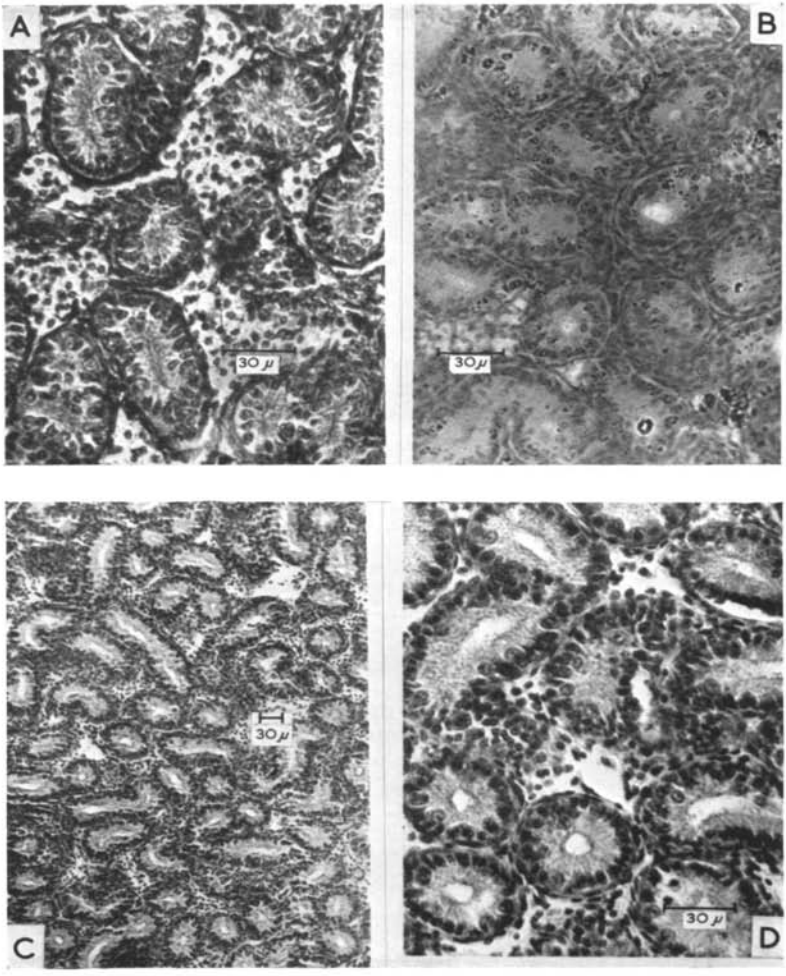

FIG. 1

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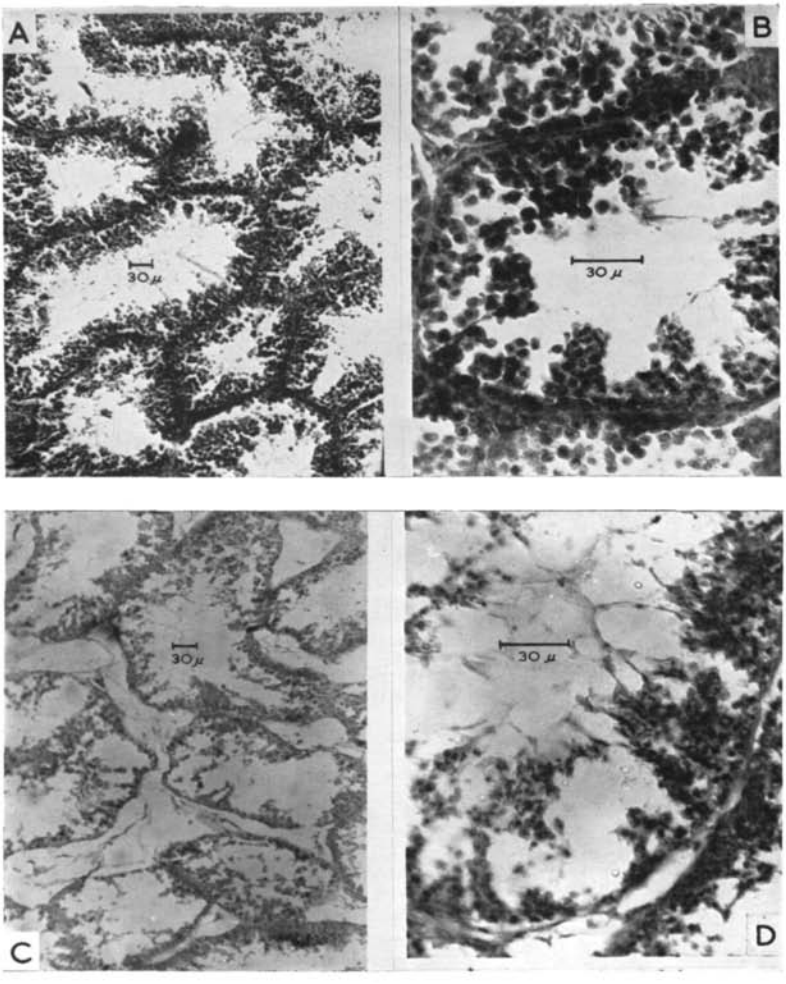

FIG. 2

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Results

At 4 weeks. Seminiferous tubules were lined for the most part with a single layer of spermatogonial cells. Primary spermatocytes were occasionally seen. In some tubules the lumen was beginning to form, and some were markedly coiled. The stroma was abundant and fibrous, full of interstitial cells (fig. 1, A).

At 8 weeks. Mitotic activity was now evident and the number of primary spermatocytes had increased. Approximately half the tubules showed a small lumen. The stroma, full of interstitial (Leydig) cells, was more abundant than at the previous age. The tubules were coiled, but the majority were still circular in cross-section (fig. 1, B).

At 12 weeks. Spermatogonial cells were more numerous and larger than before. Considerable numbers of advanced primary spermatocytes were also observed. In most tubules, the lumen was well developed. Interstitial cells were still abundant, and the stroma was extensive and fibrous (fig. 1, C, D).

At 16 weeks. The seminiferous tubules were now lined with several layers of cells: two layers of large spermatogonial cells, several layers of primary spermatocytes, and moderate numbers of secondary spermatocytes. A few spermatids were found in clusters with occasional spermatozoa among them. Sertoli cells were rare. The lumen was large and well developed in all tubules. Stroma and Leydig cells were now sparse but well formed (fig. 2, A, B).

At 20 weeks. In most regions the seminiferous tubules were now lined with 2 or 3 layers of spermatogonial cells. There were several scattered layers of primary and secondary spermatocytes, of which the secondary were the more abundant. Large numbers of spermatids were present, scattered in clusters. These were accompanied by moderate numbers of mature spermatozoa, extending towards the lumen and clumping round the scattered Sertoli cells. The lumen contained some loose spermatozoa. Most of the tubules were coiled and their cross-sections were elongated in shape. The large intertubular spaces contained only a few, well-formed, interstitial cells and but little stroma (fig. 2, C, D).

From 24 to 52 weeks. The variation in testis components in the different age-groups from 24 to 52 weeks was insignificant. The slight changes observed may indeed be due to other factors than age, such as seasonal variation in climatic or other environmental conditions. These age-groups, represented by sections at 28 weeks (fig. 3, A, B) and at 36 weeks (fig. 3, C, D), showed the highest spermatogenetic activity of all the age-groups studied. All stages of spermatogenesis were shown, with abundance of cells of all types, including ripe spermatozoa, and lumina full of spermatids and spermatozoa; the large intertubular spaces were full of well-developed interstitial cells and stroma.

It must be emphasized, however, that overlap occurs between the different phases in sections from different individuals at the same age. Cells of later

FIG. 2 (plate). Sections of testes. A, B, at 16 weeks, autopsied 22 Feb. C, D, at 20 weeks, autopsied 29 March.

stages may be observed at earlier ages in the testes of certain individuals, and some sections depart from the general average of all the individuals.

Numbers of tubules and cells. The average diameters of seminiferous tubules at 4, 8, and 12 weeks, were 44, 45 and 59 μ, respectively (see Appendix, p. 405). The numbers of tubules per square cm at the same ages were 30,125, 31,940, and 20,067, with little variation. At these ages there was no indication of cell-division; the number of cells per tubule section was approximately constant. Over the period from 16 to 24 weeks of age, however, the tubules

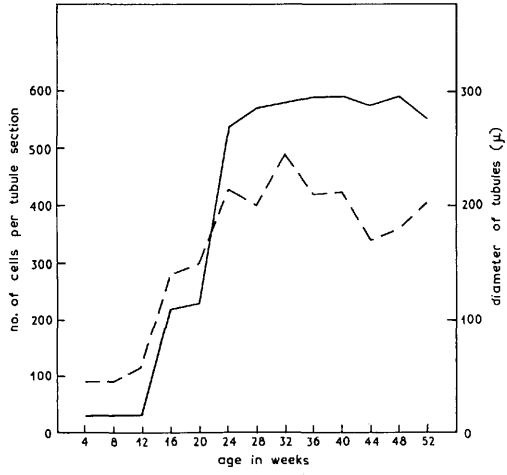

FIG. 4. Graph showing changes in the seminiferous tubules during development. Continuous line, number of cells per tubule section; broken line, diameter of tubules (μ).

increased greatly in diameter, reaching values of 139, 150, and 215 μ at 16, 20, and 24 weeks respectively. At the same time, marked spermatogenetic activity occurred, resulting in increased numbers of cells, averaging 220, 232, and 536 cells per tubule section at these ages. Owing to the increase in tubule diameter, the number of tubules per square cm decreased markedly during this period, reaching values of 2,416, 2,292, and 1,513 respectively for the three ages of 16, 20, and 24 weeks.

When tubule-diameter or cell-number is plotted as ordinate and age as abscissa (fig. 4), a typical S-shaped growth curve is obtained for both. Three phases of tubule growth can then be distinguished: in the first phase tubule diameter and cell count are apparently constant, from the fourth to the twelfth week; the second phase, from 16 to 24 weeks, coincides with the

FIG. 3 (plate). Sections of testes. A, B, at 28 weeks, autopsied 1 June. C, D, at 36 weeks, autopsied 18 Aug.

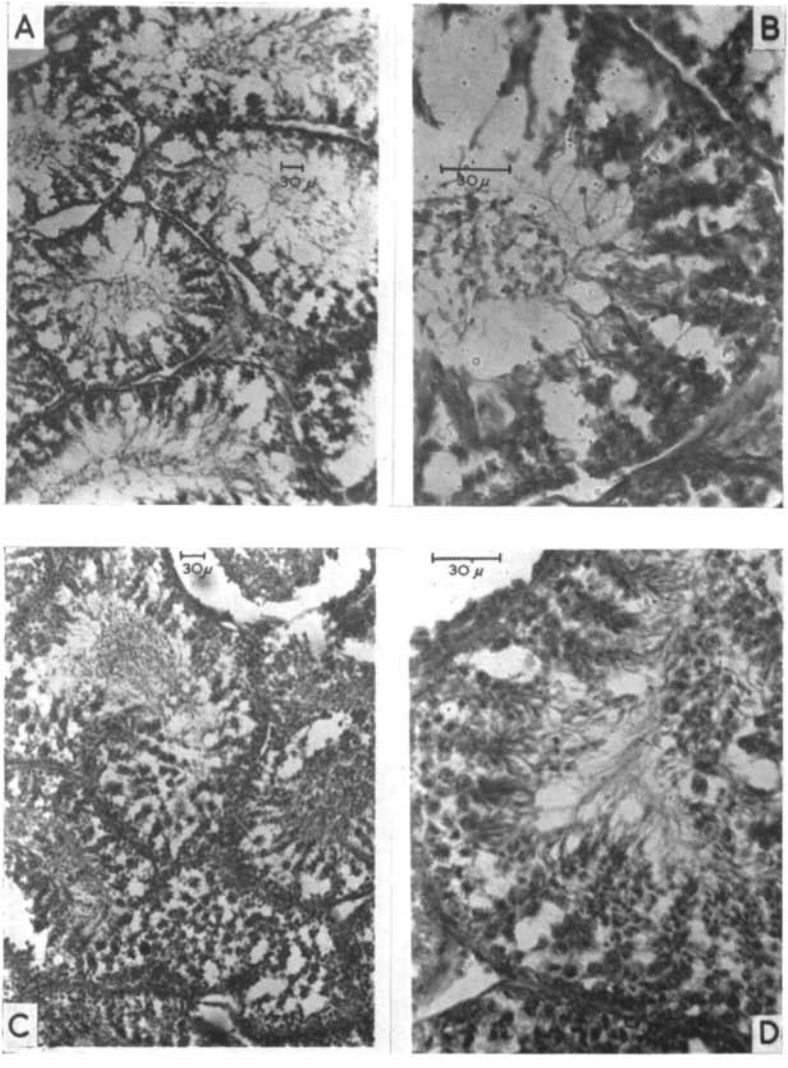

FIG. 3

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period of greatest increase in testis weight; in the third phase, from 28 to 52 weeks, maturation processes are accompanied by increase in testis weight, but there is no systematic change in tubule size and cell number.

Discussion

The period of greatest testis growth was shown to coincide with that in which the seminiferous tubules are at their largest and contain the largest number of cells per tubule section. The rapid increase in testis weight is mainly due, however, to increase in length of the tubules, rather than to increase in diameter. In other studies (Hiatt and Fisher; Kumaran and Turner; Charny, Conston, and Meranze), the formation of different types of spermatogenetic cells has been observed to occur in sequence throughout four stages. These stages were distinguished, however, by the incidence of successive stages in spermatogenesis, whereas the present study has distinguished developmental phases based on cell numbers and tubule-development. It is probable that four stages of spermatogenetic activity occur in Fayomi males as in other fowl. Phase I as defined here includes two stages of spermatogenesis, for example, while in phase II all stages of spermatogenesis are present.

APPENDIX

Histological changes in testes with age
(average of 3 males at each age)

Age in weeks	*Av. weight of pair of testes**	*No. of tubules per cm² of T.S.†*	*Av. diam. of tubule‡*	*Av. no. of cells per tubule section§*
	(*g*)		(*μ*)	
4	0·029	30,125	44	28
8	0·103	31,940	45	28
12	0·179	20,067	59	31
16	2·900	2,416	139	220
20	7·864	2,292	150	232
24	11·017	1,513	215	536
28	17·183	2,022	200	569
32	19·867	1,385	245	581
36	17·433	1,774	209	591
40	16·800	1,655	214	587
44	15·500	2,084	167	471
48	18·467	2,053	178	489
52	15·000	1,770	202	554

* Average weight of the pair of testes from one male obtained by dividing the total weight of right and left testes from three birds by three.
† Average number in 120 microscopic fields.
‡ Average of 100 seminiferous tubules.
§ Average of 120 seminiferous tubules.

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