MORPHOLOGICAL STUDIES ON THE SEXUAL MATURATION IN THE MALE JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA) I. EFFECT OF CONTINOUS LIGHTING AND CONSTANT TEMPERATURE ON THE TESTIS GROWTH

By

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Introduction

The development of the testis during the male sexual maturation has been roughly observed by the changes in the weight of this organ. The weight, however, vary with the body weight, so that the development of the spermatogenic activity should be observed histologically.

Detailed cytological and histological studies on the mammalian testis are now possible with the use of the histochemical methods such as PAS stain (1, 2). Special attention has been paid to the study of the acrosome clearly demonstrable by the PAS method, since it is related to the aquirement of the fertilizability in the spermatozoa (1-6).

It has been well known that, in birds, the male sexual maturation is closely related to the external environmental conditions. For instance, Baldwin *et al.* (7) reported that the body weight of the birds varies with seasons. However, recent progress in the technics of strict regulation of such conditions requests the re-examination of the existing data obtained in the experiments under less well-controlled conditions. No report is available on the avian sexual maturation under the strictly controlled artifitial conditions.

When the domestic fowls are considered as experimental animals, there are many differences between the avian and mammalian testis, morphologically as well as functionally (8). Moreover, the domestic fowls are not always the best as experimental animals, since they are too large in size and require too long a time for growth. Besides, in domestic fowls, the changes of the male sexual

maturation is less sensitive to the seasonal changes than those of the birds less domesticated (9).

My preliminary observation (10) showed that the Japanese quails are quite suitable as experimental animals because of their smaller size, rapid growth and higher sensibility to the environmental factors. In spite of the increasing importance of the Japanese quails, extensively reared in the Tokai district recently, there is no report on the sexual maturation of the male birds under controlled conditions.

The present study was planned, therefore, to investigate the sexual maturation in the male Japanese quail from hatching to 40 days of age under continous lighting and constant temperature, and to provide detailed informations on the sexual maturation of this species as the first step of the investigations concerning the sexual maturation of the male Japanese quail.

Materials and methods

The Japanese quail chicks purchased from one hatchery in Toyohashi City were used for this study. At the beginning of the experiment, 240 birds were maintained in 12 cages. Each cage contained 20 birds, selected to give the same mean body weight in each cage. Koitotron, an air conditioning chamber (Type E-A, Koito Industry Co. Ltd., Fig. P-1) was used for the feeding chamber in this study.

The light and temperature were controlled as follows. According to my preliminary experiment, the temperature was set at 38°C at the beginning and then lowered to 30°C on the fifteenth day of age. Thereafter the temperature was maintained at 30°C to the end of this experiment. The measurement of the temperature was done by an autorecording thermometer placed in the middle of the chamber. For light, the chamber was illuminated continously by four fluorscent lamps fixed for each cage. The birds were fed twice a day in the morning and in the evening.

Testis samples were collected from 12 birds killed every five days after hatching. After the body weight was weighed, the testis were weighed with a directindi-cating balance. One hundred and eight testes were fixed in Zenker-formol solution (Helly's fluid) for the histological study. They were embedded in paraffin and sectioned at 5 μ. One hundred and eight cross sections of the middle part of the organs were stained with PAS-hematoxylin. The diameter of the seminiferous tubules, and the size of the germ cells and their nuclei were measured by an ordinary micrometer with these sections.

Results and Discussion

1. The body weight.

The body weight of the 108 birds from hathcing to 40 days of age was tested by the formula of CMNPHOB (11). Consequently one bird (body weight 86 g,

30 days of age) was omitted. The body weight of the remaining 107 birds are shown in Table 1 and Figs. 1-2. The increase of body weight was moderate up to 10 days of age. It became remarkable after this period up to 35 days of age, after which period it became again moderate.

Table 1. Relation between body weight (g) and days of age.

No. of individuals	Age in days								
	0	5	10	15	20	25	30	35	40
1	6.2	11	19	21	44	57	57	82	97
2	6.0	13	16	27	43	62	—	86	85
3	6.2	11	12	27	45	56	68	90	84
4	6.2	11	16	35	45	60	67	87	92
5	6.5	15	14	29	37	65	62	86	91
6	6.3	12	10	32	37	60	48	85	83
7	6.9	12	13	35	38	49	74	87	86
8	6.9	11	13	30	50	57	78	87	85
9	6.9	13	14	25	46	58	80	80	86
10	5.1	12	13	28	36	56	61	77	93
11	6.3	12	12	27	52	48	69	76	86
12	6.3	12	12	35	40	54	68	83	88
Mean	6.33	12.1	13.7	29.0	42.8	56.8	66.5	83.8	88.0
Standard deviation	±0.39	±1.16	±2.39	±4.58	±5.24	±4.89	±10.59	±4.32	±4.28

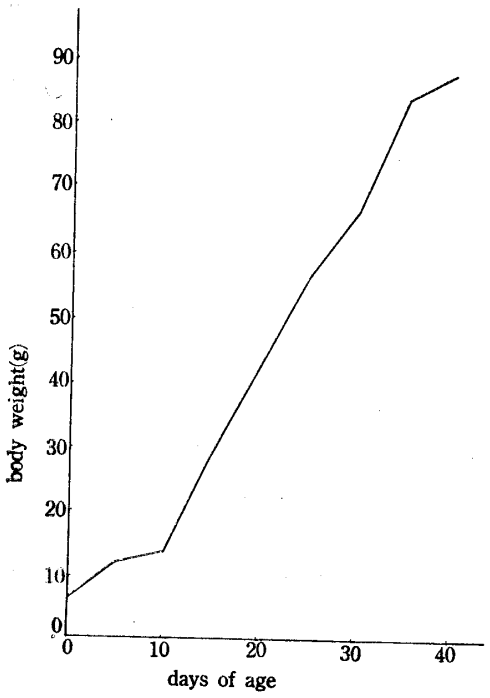

Fig. 1. Relation between the average body weight and days of age.

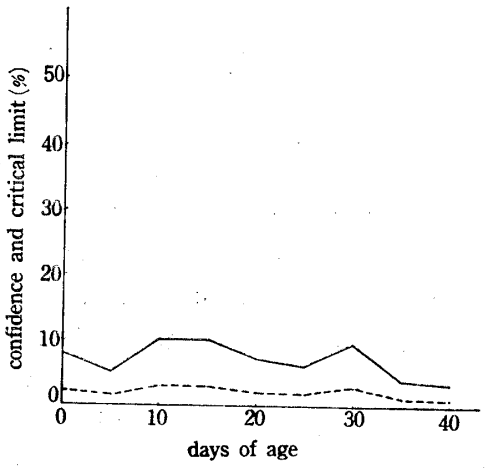

Fig. 2. Confidence and critical limits of body weight at various days of age.

As shown in Fig. 1 and 2, the curve of the body weight increase during the growth of the quails was approximately sigmoid, as expected in the case of the growth curve. The individual variations in the body weight were relatively large at 10 to 20 days of age, and at 30 days of age. However, the percentages of critical limit (5% of significance level) to the average body weight were less than 11 percent in most cases. This indicates that the individual variation of the weights was generally moderate in this experiment.

2. The testis weight.

The weight of the bilateral testes at each period are presented in Table 2. The relation between the day of age and the average weight of testis are shown

Table 2. Relation between testis weight (mg) and days of age.

No. of individuals	Age in days								
	0	5	10	15	20	25	30	35	40
1	0.5	0.7	3.4	5.0	82.7	51.5	148.4	916.7	1307.0
2	0.5	0.4	3.1	6.8	80.9	158.9	—	1116.7	835.6
3	0.6	0.4	2.3	7.2	49.4	221.9	318.2	1192.8	1827.8
4	0.6	0.8	3.6	28.5	109.8	138.0	58.1	967.6	1609.2
5	0.5	0.8	2.9	7.2	35.6	127.7	281.7	1162.9	1816.3
6	0.5	0.5	5.2	10.3	26.2	91.6	34.2	1033.8	1151.7
7	0.5	0.7	3.0	29.7	36.4	62.3	408.8	1486.9	1782.4
8	0.6	0.4	2.3	9.5	17.8	65.1	380.5	992.8	1901.1
9	0.6	0.7	1.6	11.9	92.0	81.3	586.4	799.8	1108.6
10	0.4	0.6	2.0	6.0	35.9	83.1	194.3	1190.7	2212.0
11	0.3	0.7	3.4	6.0	141.6	18.0	236.6	664.2	936.1
12	0.5	0.5	3.1	10.2	79.2	126.9	163.6	1520.0	1075.6
Mean	0.5	0.6	3.0	11.5	65.6	102.2	255.6	1087.1	1463.8
Standard deviation	±0.09	±0.16	±0.93	±8.47	±37.99	±42.31	±162.84	±756.31	±446.15

in Fig. 3. The results obtained statistically are given in Fig. 4. The individual deviation of the testis weight at each period was greater than those of the body weight. The increase of the testis weight from hatching to 15 days of age was markedly large, and the increase from 30 to 35 days of age was most prominent. According to Breneman (12), the testis of the domestic fowls grow uniformly from hatching to about 55 days of age. Thereafter to 63 days of age, however the standard errors of the weight variation became large, when the pituitary growth became remarkable. It may be supposed that, in the present study, the marked variation of the testis weight was caused by the variation in the rate of the pituitary growth of the birds under continous lighting.

The testis weight relative to body weight is presented in Fig. 5. It indicated that the growth of the testis became not parallel with the growth of body after

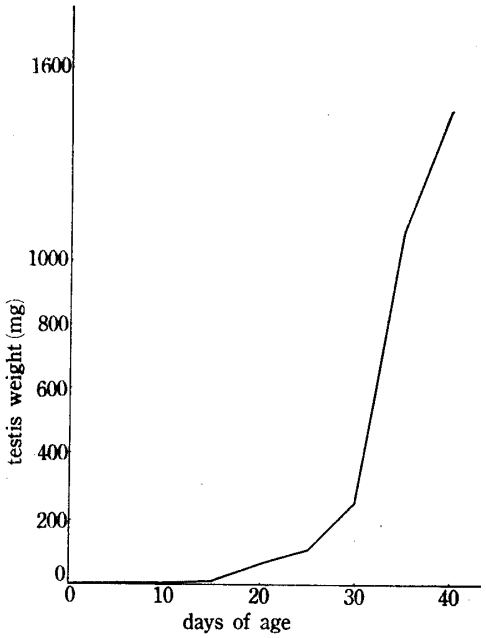

Fig. 3. Relation between average testis weight and days of age.

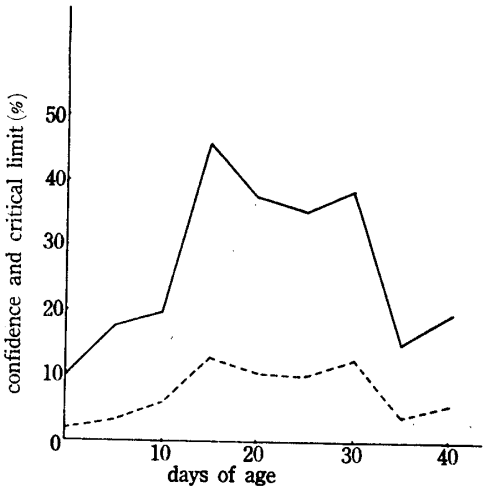

Fig. 4. Confidence and critical limits of testis weight at various days of age.

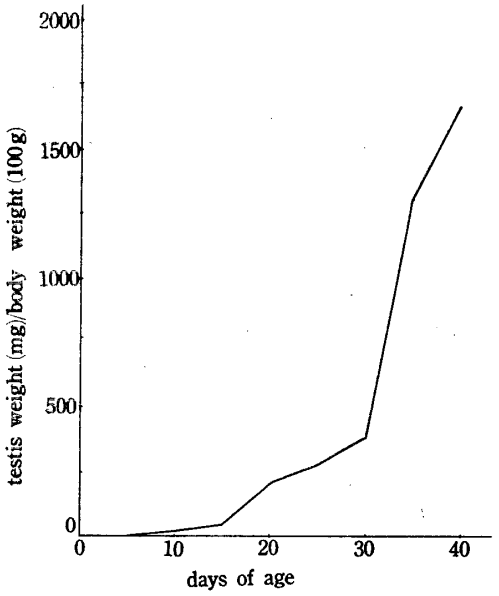

Fig. 5. The average testis weight per 100 mg of the body weight.

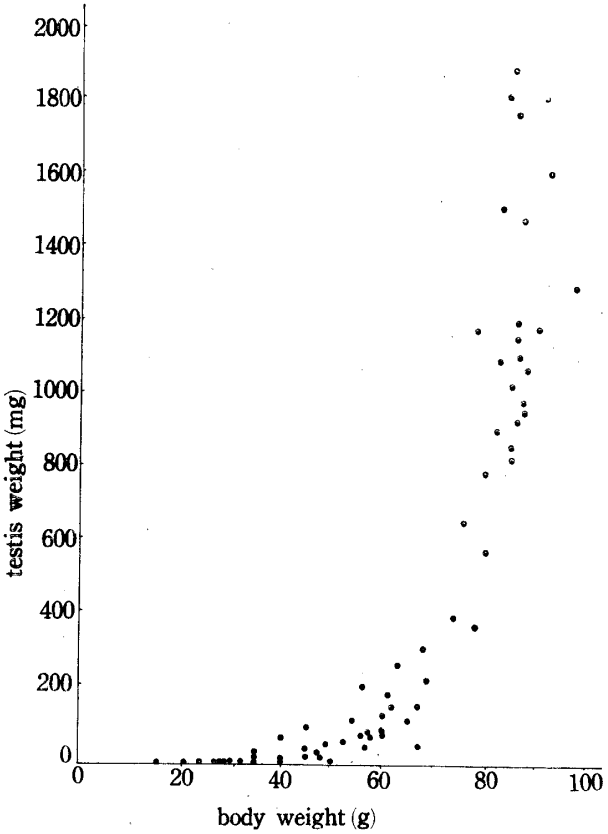

Fig. 6. Relation between the testis weight and body weight regardless of the age in days.

20 days of age. As the testis weight still increased between 35 and 40 days of age, the sexual maturation was not yet completed. The relation between the body weight and the testis weight, regardless of age, is given in Fig. 6. The relation between the two weights was closer than the relation between the testis weight and age. It was evident that the testis grew very rapidly when the body weight exceeded 60 g.

In the Japanese quail, it was obvious that the growth of the testis was strongly promoted at the period from 15 to 30 days of age, and that the male sexual maturation in this species was not completed at 40 days of age. It may be noted that there was a closer relation between body weight and testis weight.

3. The histology of the testis growth.

The development of the spermatogenic activity in the testis, associated with the sexual maturation, was observed by the changes of the histological picture of this organ. The results are as follows:

(a) The diameter of the seminiferous tubules.

It has been observed that the growth of the testis occurs as the results of the elongation, branching and enlargement of the semiferous tubules. In cockerels, a close relation between the diameter of the seminiferous tubules and the weight of the comb was observed (13). The size of the tubules was closely related to the process of the testis maturation in the rat (14). In this study, therefore, the diameter of the tubules in the quails were measured as follows: The mean diameter of 20 tubules of each bird were measured with the microphotographs of the histological preparations, taken at the same magnification (×400).

Table 3. Relation between the diameter of the seminiferous tubules (μ) and days of age.

No. of individuals	Age in days								
	0	5	10	15	20	25	30	35	40
1	16.6	19.6	35.9	31.7	108.8	74.1	117.1	210.5	193.4
2	21.4	23.4	29.8	38.1	88.2	111.1	—	198.4	166.4
3	16.0	26.1	29.2	37.4	68.9	123.8	126.0	184.9	232.7
4	19.4	21.0	35.9	49.2	85.2	99.3	74.9	195.7	203.1
5	15.1	27.6	34.8	30.7	69.6	96.4	146.8	193.4	216.4
6	15.7	22.3	31.7	32.3	33.4	78.6	68.2	197.9	213.5
7	14.9	22.7	34.8	84.5	72.6	70.4	157.9	234.9	206.8
8	16.8	14.8	28.8	40.6	61.5	77.8	146.0	203.8	229.0
9	16.2	23.7	27.1	43.4	94.1	71.9	179.4	188.3	204.6
10	14.5	18.4	30.1	29.9	55.6	95.6	112.7	200.9	222.4
11	17.3	22.5	24.2	35.3	80.2	50.4	106.7	179.4	184.6
12	16.3	22.8	27.2	35.9	92.6	106.7	107.5	210.5	188.3
Mean	16.7	22.1	30.8	40.8	75.2	88.0	120.3	199.9	205.1
Standard deviation	±1.96	±3.30	±3.59	±14.88	±19.14	±20.43	±35.22	±14.52	±19.48

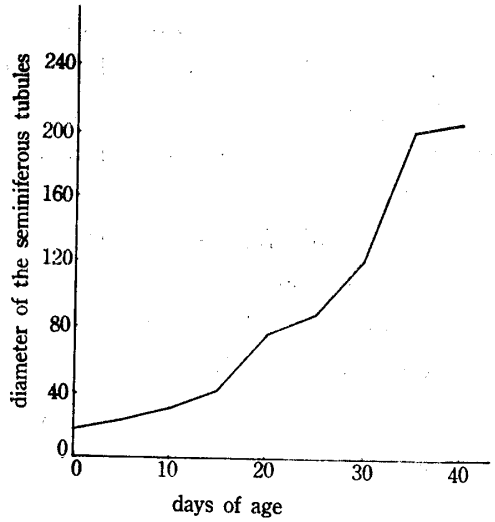

Fig. 7. Relation between the diameter of the seminiferous tubules and days of age.

The mean diameter of the seminiferous tubules at various days of age are presented in Table 3. The relation between the age and the mean diameter of the tubules are also showed in Fig. 7. Generally the diameter increased with the growth of the birds. It was relatively small before 15 days of age. The increase from 30 to 35 days of age was remarkable, but after 35 days of age it was nearly negligible (Figs. 9-17). On the other hand, a close relation was observed between the diameter of the seminiferous tubules and the testis weight. The increase of the diameter in the testis weighing less than 400 mg was large, but small in those weighing more than 500 mg. Therefore, the increase of the testis weight up to 500 mg resulted mainly from the enlargement of the seminiferous tubules, and thereafter it was done not by the enlargement, but by the branching and elongation of the seminiferous tubules in the testis weighing more than 500 mg.

(b) The number of the seminiferous tubules.

The growth of the seminiferous tubules was observed by counting the number

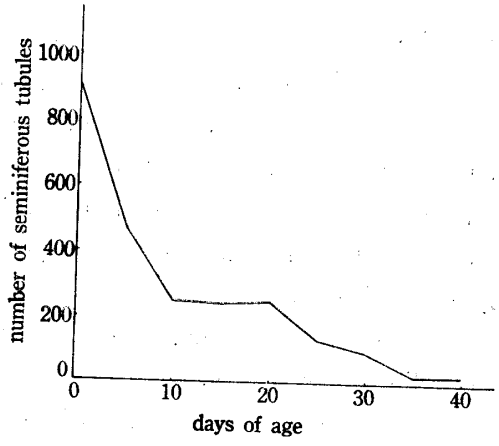

Fig. 8. Average numbers of the seminiferous tubules in the limited area of the sections and the days of age.

of the tubules in a limited area (Fig. 8). The number remarkably decreased with the advancing days of age. The number of the tubules became very small when the diameter exceeded 150 μ at 35 days of age. This indicated that the number of the seminiferous tubules in the limited area was reverse to the degree of their growth (Figs. P2-P10).

(c) Relative proportion of the seminiferous tubules to the interstitial tissues.

The proportion of the seminiferous tubule parenchyme in the limited area was measured with the microphotographs of the testis tissues in the two birds of each period. The results are presented in Figs. P2-P10 and 9. The proportion

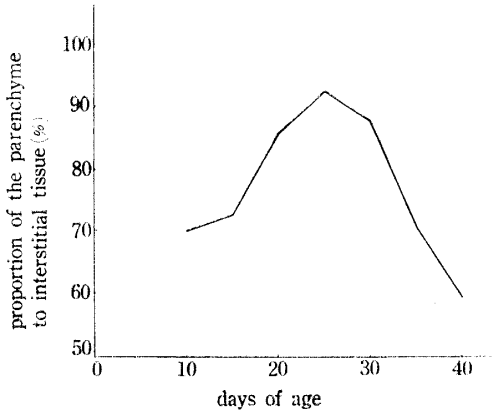

Fig. 9. Proportion of the parenchyma of the seminiferous tubule to the interstitial tissue (%).

of the interstitial cells were large in the earlier period of post-hatching. They decreased gradually in the later periods. The proportion of the parenchyme reached to 92 percent at 25 days of age accompanied with the remarkable enlargement, branching, and elongation of the seminiferous tubules. After 30 days of age the relative proportion of the parenchyme decreased again, due to the increase of artifitial gaps resulting from the shrinkage of the seminiferous tubules during fixation.

4. Spermatogenic activities.

(a) The sequense of the development of the spermatogenic cells.

Various stages of the spermatogenesis were observed in the testis of 40 days of age, following the work of Clermont *et al.* (15) in the rat. The sizes of the germ cells in the fixed preparations are presented in Table 4. The four stages of the spermiogenesis were clearly differentiated by the presence of the acrosomic system demonstrable by the PAS method. The acrosome granules were found somewhat apart from the nuclei in the preparation fixed by Helly's fluid.

The seminiferous epthelia generally consisted of four to five layers of germ cell generations, and the combinations of the generations appeared, as reported in the mammals (16, 17). The details of the combination will be reported at another opportunity.

Table 4. The sequence of spermatogenesis.

Cell species	No.	Phase	Diameter (μ)		Cytological events
			Nucleus	Cytoplasm	
Nurishing cell	Se	Sertoli cell	6.5×6.5	—	Cytoplasm not clearly distinguished
Primordial germ cell	S	Supporting cell	4×8	6×9	Nucleus with a large nucleolus
	G	Gonocytes	7×11	9×14	Light, round nucleus
Spermatogonia	1	Spermatogonia A	4×6.5	5×9	Elongated nucleus with fine chromatin
	2	Spermatogonia B	5.5×6.5	6.5×8	Round nucleus with coarse chromatin
Primary spermatocytes	3	Resting	5×5	6×7	Small, round nucleus
	4	Leptotene to Zygotene	5.5×5	6.5×7.5	Increase of cell volume and condensation of chromatin
	5	Early pachytene	5×6.5	8.5×11.5	ibid.
	6	Late pachytene	6×7	8×15	Largest germ cell 1st division
Secondary spermatocytes	7	Secondary spermatocytes	4×5	6.5×7.5	Strongly stained nuclear membrane. 2nd division
Spermatids	8	Golgi	3.5×3.5	7×7	No acrosomic granules
	9	〃	3.5×3.5	5×5	Appearance of acrosomic granules
	10	〃	3.5×3.5	5×5	Application of acrosomic granules to nucleus
	11	Head cap	3×3.5	4.5×7	Beginning of flattening of nucleus. Formation of head cap
	12	〃	2.5×3.5	5.5×5.5	Darkening of nucleus
	13	〃	2.5×3.5	5×6.5	Further darkening and flattening of nucleus
	14	Acrosome	2.5×4.5	5.5×6.5	Completely darkened nucleus
	15	〃	1.5×7	—	Elongation of nucleus
	16	〃	1×9	—	Formation of sperm head like nucleus
	17	Immature sperm	0.5×13	—	Immature sperm still attached to Sertoli cell
	18	〃	0.3×29	—	Migration of immature sperm into tubular lumen
Spermatozoa	19	Mature sperm	—	—	Mature sperm in lumen

(b) The spermatogenic stages in the seminiferous tubules.

For the study of the spermatogenesis, 20 seminiferous tubules were observed on one bird of each stage. The most advanced type of the germ cells was recorded as indicators of the spermatogenesis in the tubules.

(i) Relation between the days of age and the spermatogenesis.

As shown in Fig. 10, the seminiferous epithelia were entirely occupied by the gonocytes up to 15 days of age (Figs. P-11). At 20 days of age the appearance of the spermatogonia as well as the primary spermatocytes was noted. A mucin-

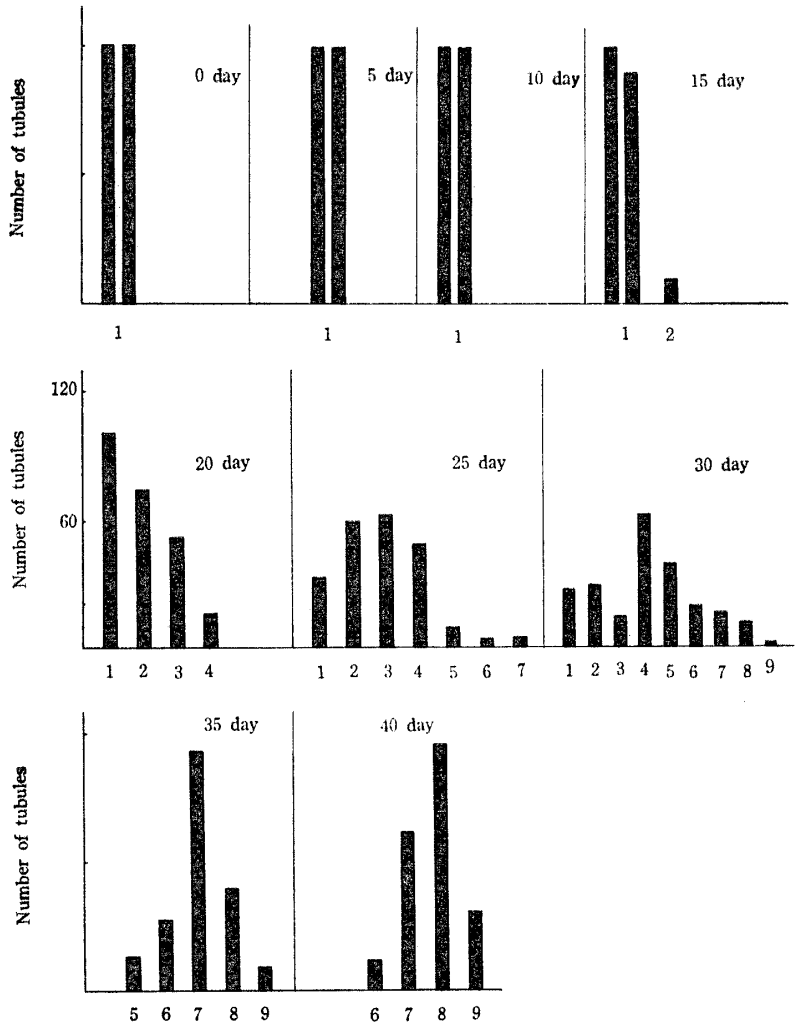

Fig. 10. Relation between the development of the spermatogenesis and the days of age.

Remarks: 1 indicates primordial germ cells; 2 spermatogonia; 3 leptotene to zygotene primary spermatocytes; 4 pachytene primary spermatocytes; 5 Golgi phase spermatids; 6 head cap phase spermatids; 7 acrosome phase spermatids; 8 immature spermatozoa; 9 mature spermatozoa.

like, PAS positive substance in the lumens of the tubules in the earlier periods disappeared at the same time. Mature spermatozoa appeared at 30 days of age (Figs. P-12, P-13). This suggested that at 30 days of age the spermatogenesis may be stimulated definitely, since all stages of germ cells were observed at this period. After 35 days of age no gonocytes remained within the seminiferous tubules.

(ii) The relation between the diameter of the seminiferous tubules and the spermatogenesis.

The relation between the diameter of the seminiferous tubules and the spermatogenesis are presented in Fig. 11. The tubules of more than 90-120 μ in diameter contained no gonocytes, indicating that in such tubules the spermato-

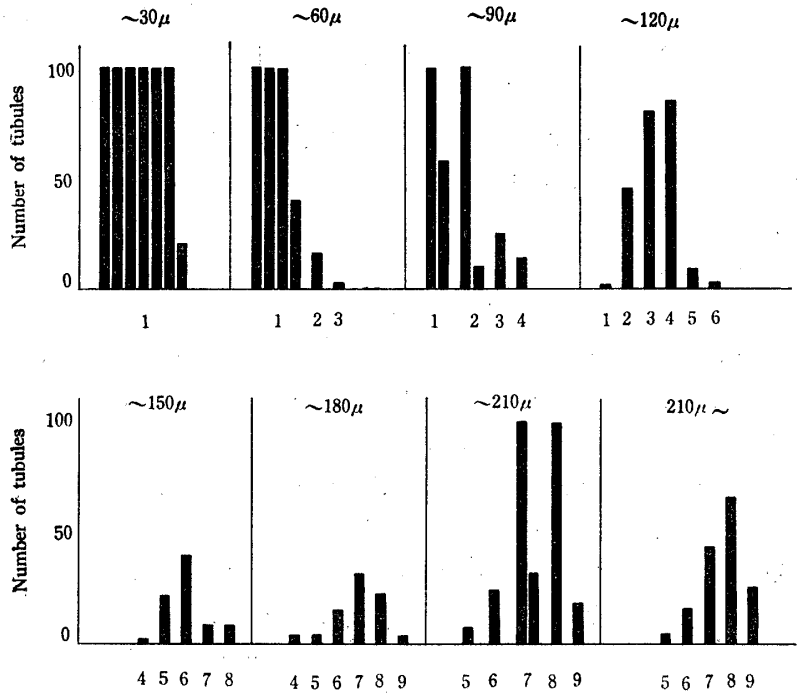

Fig. 11. Relation between the development of the spermatogenesis and the diameter of the seminiferous tubules.

Remarks: 1 indicates primordial germ cells; 2 spermatogonia; 3 leptotene to zygotene primary spermatocytes; 4 pachytene primary spermatocytes; 5 Golgi phase spermatids; 6 head cap phase spermatids; 7 acrosome phase spermatids; 8 immature spermatozoa; 9 mature spermatozoa.

genesis started. The tubules that contained mature spermatozoa were more than 180-210 μ in diameter. The results in this section together with those in the previous one showed that the period of 90-120 μ and the period of 180-210 μ in tubules diameter corresponded to the period of 25-30 days of age and 35 days of age, respectively. It was clear that, at 30 days of age, the sexual maturation in the male Japanese quail was stimulated significantly.

Conclusion and Summary

The sexual maturation in the male Japanese quail (*Coturnix coturnix japonica*) under continous lighting and constant temperature was studied morphologically. The results are summarized as follows:

1. During from hatching to 15 days of age, the weight of the testis increased moderately. The small seminiferous tubules contained PAS positive mucin-like substances, but no differentiated germ cells such as spermatogonia and spermatocytes. This indicates that the testis is still undeveloped.
2. The weights of the testis increased rapidly after 15 days of age, concomitant with the enlargement of the seminiferous tubules and the appearance of the spermatogonia and spermatocytes. This suggests that the maturation of the

testis was stimulated, at this period, probably through the anterior pituitary hormones. Individual variation of the testis development observed statistically at this period suggests that the time of the initiation of the hypophyseal stimulation on the testis growth differs in the individual birds considerably.

3. The extremely enlarged seminiferous tubules of the testis from 25 to 35 days of age contained a series of the spermatogenic cells from the spermatogonia to the mature spermatozoa.

4. The increase of the testis weights became relatively small after 35 days of age. The seminiferous tubules shows no increase in diameter. This indicates that the growth of the testis was mainly done by the elongation and branching of the seminiferous tubules after 35 days of age.

5. The above results show that the sexual maturation in the male Japanese quail under continous lighting started at as early as 15 days of age and was almost finished at 35 days of age. The results on the sexual maturation of the birds under shorter lighting will be reported in the next paper, together with the discussion on the effect of lighting on the maturation.

Acknowledgement

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References

1) Leblond, C. P. and Y. Clermont (1952). Amer. J. Anat., **90**, 167.
2) Cavazos, L. F. and R. H. Melampy (1954). Amer. J. Anat., **95**, 467.
3) Onuma, H. Y. Nishikawa and H. Kudo. (1963). Bull. Nat. Inst. An. Indust. (Chikushikempo), **1**, 135. (in Japanese).
4) Onuma, H. (1963). *ibid.* **2**, 209.
5) Onuma, H. and Y. Nishikawa (1963). *ibid.* **1**, 125.
6) Austin, C. R. and M. H. Bishop (1958). Pro. Roy. Soc. B., **149**, 241.
7) Baldwin, S. P. and S. C. King (1938). Auk., **55**, 416.
8) Sturkie, P. D.(1954). Avian Physiology. Comstock Publishing Associates. Ithaca, New York.
9) Suzuki, Y. (1956). Endocrinol. Symposium (Naibumpitsu no Tsudoi). Edited by Y. Ito. Kyodoisho, Tokyo. Vol. **8**, 101.
10) Yamamoto, S. (1963). Unpublished.
11) Masuyama, M. (1953). Statistics in Small Number. (Shosurei no Matomekata) Kawade, Tokyo. (in Japanese).
12) Breneman, W. B. (1944). Endocrinology, **36**, 456.

13) Yamauchi, S. (1954). Bull. Naniwa Univ. Ser. B., 101.
14) Homma, K. (1954). Medicine and Biology (Igaku to Seibutsugaku) **31**, 276. (in Japanese).
15) Clermont, Y. and C. P. Leblond (1961). Amer. J. Anat. **96**, 229.
16) Perey, B., Y. Clermont and C. P. Leblond (1961). Amer. J. Anat., **108**, 47.
17) Roosen-Runge, E. C. (1955). Anat. Rec., **132**, 385.

Plate 1

Explanation of Photographs

Materials used were the testes of the Japanese quail chicks, fixed in Helly's fluid and sectioned at 5 μ in paraffin. All microphographs except Fig. P-1. were taken with the cross sections stained by PAS-hematoxylin, at ×400 (Figs. P-2-P-10), at ×1000 (Figs. P-11, P-12), or at×1600 (Fig. P-13).

Fig. P-1. The Koitotron, the air conditioning chamber for feeding experimental animals, used in this study (Type E-A, Koito Industry Co. Ltd., Tokyo). Its front view.

Fig. P-2. Testis of the day of hatching (0 day of age). Small seminiferous tubules with no visible lumen occupied the entire testis.

Fig. P-3. Testis of five days old bird. Small tubules contained no spermatogonia. The interstitial tissues were relatively abundant.

Fig. P-4. Testis of 10 days old bird. Small seminiferous tubules contained a mucin-like PAS-positive substance and degenerating gonocytes.

Fig. P-5. Testis of 15 days old bird. The seminiferous tubules remained in the immature state, with no differentiated spermatogenic cells, with a slight decrease of the mucin-like substance and enlargement of the tubules, a clear luminal space appeared.

Fig. P-6. Testis of 20 days old bird. The majority of the seminiferous epithelia were still occupied by the gonocytes and supporting cells. but some consisted of spermatogonia and primary spermatocytes.

Fig. P-7. Testis of 25 days old bird. Remarkable enlargement and elongation of the seminiferous tubules were noted. The seminiferous tubules contained abundant spermatocytes at the pachytene phase, but few gonocytes.

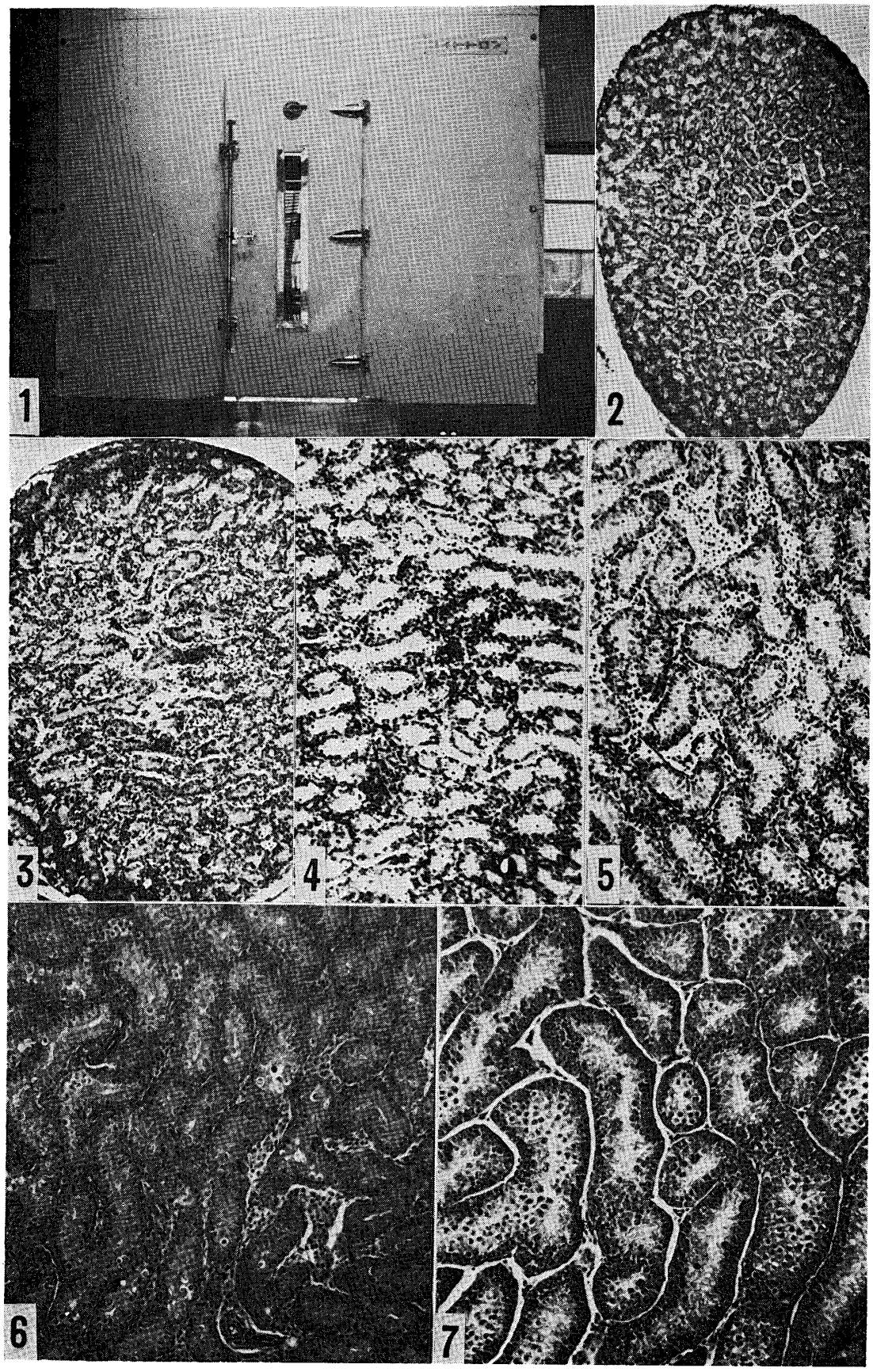
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Plate 2

Explanation of Photographs

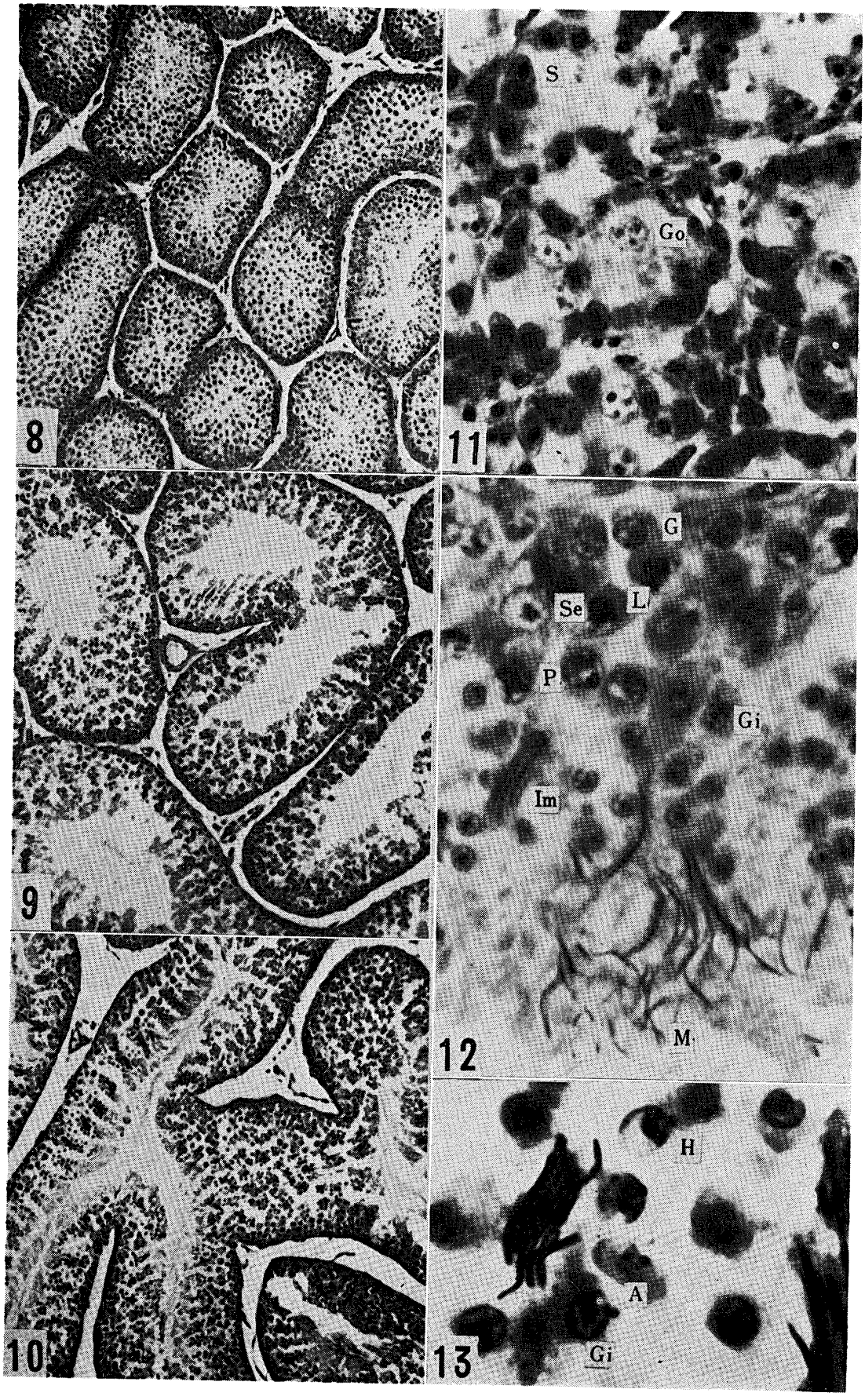
Fig. P-8. Testis of 30 days old bird. Large tubules contained generations of spermatogenic cells. The spermatids of Golgi and Head cap phases were numerous.

Fig. P-9. Testis of 35 days old bird. The extremely large seminiferous tubules contained immature spermatozoa associated with a Sertoli cell cytoplasm. The interstitial cells were only a few in number.

Fig. P-10. Testis of 40 days old bird. No enlargement of the seminiferous tubules occured after 35 days of age. The branching of the tubules were so remarkable that only three tubules were seen. The mature type of spermatozoa was seen in the lumen of the tubules.

Fig. P-11. Immature type of the seminiferous tubules (15 days ofage). In small tubules, large round gonocytes (Go) were located centrally and elongated supporting cells (S) peripherally. Fibloblast-like interstitial cells occupied intertubular spaces.

Figs. P-12, P-13. Mature type of the seminiferous tubules (30 days of age). In large tubules, various stages of spermatogenic cells were seen : Mature spermatozoa (M), Immature spermatozoa (Im), Head cap phase spermatids (H), Golgi phase spermatids (Gi), Pachytene primary spermatocytes (P), Leptotene-Zygotene primary spermatocytes (L), B type spermatogonia (G), Sertoli nucleus (Se), and Acrosomic granule (A).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