QUANTITATIVE ANALYSIS OF SPERMATOGENESIS IN RATS.

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The Science of spermatology is nearly three hundred years old. Various workers Brown (1885), Benda (1887), Von Ebner (1888), Lenhossek (1898), Regaud (1900), Schoenfeldt (1902), Weldeyar (1906), Hoof (1912), Roosen Runge (1950), and Leblond and Clermont (1952) have attempted to identify different stages of spermatogenesis from time to time; but none made an attempt to study spermatogenesis on a quantitative basis. Quantitative studies on spermatogenesis were first undertaken by Roosen Runge in 1950. This method has, however, not been subjected to proper evaluation so far. It is intended to study the same in normal albino rats (Kasauli Strain) under controlled conditions.

METHODS AND MATERIAL

Normal male albino rats weighing between 150-200 grams were selected and kept on the synthetic diet having 20% casein for the period of 30 days. Those rats which showed any sign of disease or loss in body weight were not included in the series. At the end of the experimental period the rats were weighed and sacrificed under light ether anaesthesia. The testes were removed and examined macroscopically. For histological studies they were fixed in the Helly's fluid, sections of 3-4 microns were cut from the mid portion of both the testes of each rat and stained by "Per Iodic Acid Fuchsin Sulfurous Acid" technique (Leblond & Clermont 1952). Quantitative analysis of spermatogenesis, based on Roosen Runge (1950) classification, was undertaken.

OBSERVATIONS

Observations regarding the gain in body weight during this period, and the size and weight of the right and left testes in these animals are given in the table below.

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TABLE I

S. No.	Initial body wt. Gm.	Final body wt. Gm.	Gain in body wt. Gm.	Size of testes Rt. m.m.	Size of testes Lft. m.m.	Average size. m.m.	Wt. of Testes Rt. Gm.	Wt. of Testes Lft. Gm.	Average wt. Gm.
1	2	3	4	5	6	7	8	9	10
1.	150	170	20	11×13	11×14	11 ×13.5	0.78	0.82	0.80
2.	165	193	28	12×15	12×16	12 ×15.5	0.92	1.01	0.96
3.	162	185	23	14×16	14×16	14 ×16	0.98	0.91	0.94
4.	152	173	21	11×13	12×14	11.5×13.5	0.82	0.95	0.88
5.	164	180	16	12×15	12×16	12 ×15.5	1.06	1.12	1.09
6.	152	174	22	10×12	12×15	11 ×13.5	0.82	0.82	0.85
Average.	157.5	177.5	20	12×14	12×15	12 ×14.5	0.91	0.97	0.94

The gain in the body weight in this series ranged from 16 grams to 28 grams per rat. It was also observed that the size and the weight of the left testis showed on an average higher values than the right. The testes were pale pink in colour and soft in consistency.

Main characterstics utilised for differentiating the various stages of spermatogenesis are represented in figures 1 to 8 from animal No. 5 of the normal series.

Results of the quantitative studies of a total of 2,400 tubular cross sections are depicted in the table below.

TABLE II

Stages. S. No.	I	II	III	IV	V	VI	VII	VIII
1.	4.0%	6.0%	13 5%	7.0%	9.0%	33.5%	12.0%	15.0%
2.	4.6 ,,	4.1 ,,	15.7 ,,	5.0 ,,	10.4 ,,	32.0 ,,	11.7 ,,	16.5 ,,
3.	4.0 ,,	5.3 ,,	12.0 ,,	4.7 ,,	8.5 ,,	36.5 ,,	13.0 ,,	16.0 ,,
4.	4.9 ,,	3.6 ,,	13.4 ,,	6.7 ,,	7.6 ,,	32.9 ,,	13.7 ,,	17.2 ,,
5.	2.6 ,,	5.4 ,,	15.2 ,,	4.6 ,,	9.6 ,,	33.7 ,,	11.3 ,,	14.1 ,,
6.	3.5 ,,	4.6 ,,	13.7 ,,	4.7 ,,	8.1 ,,	35.6 ,,	12.7 ,,	17.6 ,,
Average	3.9%	4.8%	13.9%	5.4%	8.8%	34.0	12.6%	16.0%

The average values of different stages of this series are represented in Graph I.

Average percentages of various stages in the normal control group are arranged below in their order of frequency and are compared with the average percentages of stages derived by Roosen Runge (1950) in his control group of normal rats.

TABLE III.

Stage.	Present value. %	Roosen Runge. %	Range. %
VI ..	34.0 ..	33.6 ..	30-40
VIII ..	16.0 ..	17.6 ..	16-18
III ..	13.9 ..	14.5 ..	14-16
VII ..	12.6 ..	11.6 ..	11-13
V ..	8.8 ..	9.4 ..	8-10
IV ..	5.4 ..	4.8 ..	4 - 5
II ..	4.8 ..	4.8 ..	4 - 5
I ..	3.9 ..	3.7 ..	3 - 4

Analysis of these results shows that the average percentages of the various stages in the present series corresponded more or less with the percentages of stages obtained by Roosen Runge.

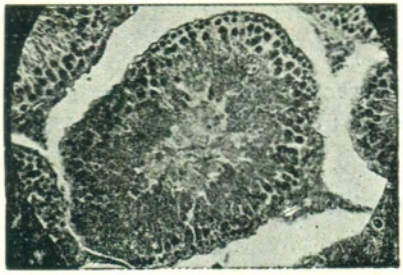


Fig. 1.

Stage I : Absence of spermatozoa; presence of several layers of spermatocytes (spermatogonia-B) & layers of large spermatocytes.

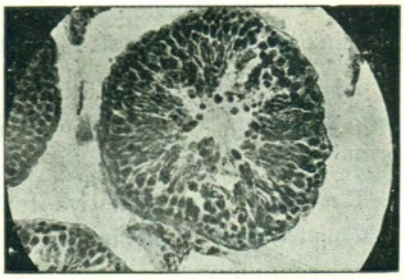


Fig. 2.

Stage II : Elongation of spermatids nuclei and their formation into bundles. Small spermatogonia and spermatocytes noticed at the periphery.

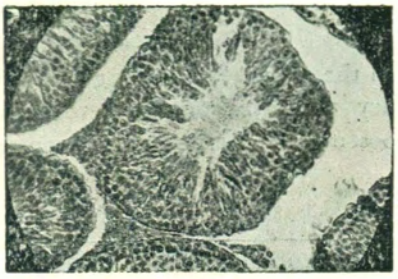


Fig. 3.

Stage III : Spermatids defenitely form into bundles. The primary spermatocytes are largest in this stage.

Fig. 4.

Stage IV : Spermatogonia not clearly visible primary (large) and secondary (small) spermatocytes division in groups; spermatids bundles show tendency of migration from the lumen towards the periphery.

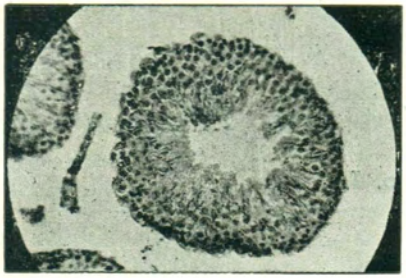


Fig. 5.

Stage V : Spermatids bundles seen deep in the wall of the tubules, small dark prespermatocytes visible. Spermatogonia shows very faint out-line and does not show any mitotic division.

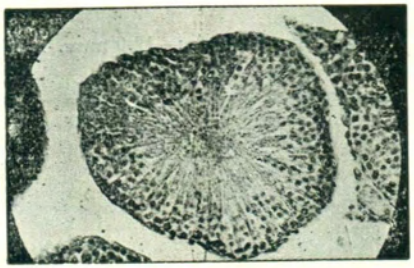


Fig. 6.

Stage VI : The "Wheel Spoke" appearance; spermatogonia of type 'B' seen; spermatozoa penetrates the wall of the tubules.

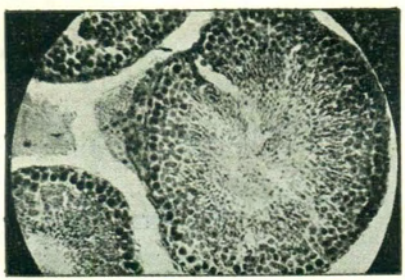


Fig. 7.

Stage VII : Spermatozoa have ieft the periphery, hardly any bundle seen. Spermatogonia forms a complete outer layer.

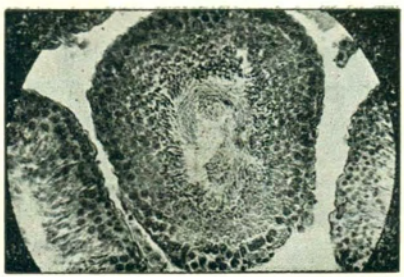


Fig. 8.

Stage VIII : Spermatozoa line the lumen; their tail forms a characterstic vortex. The dark granules peripheral to the head of the spermatozoa represent cellular debris.

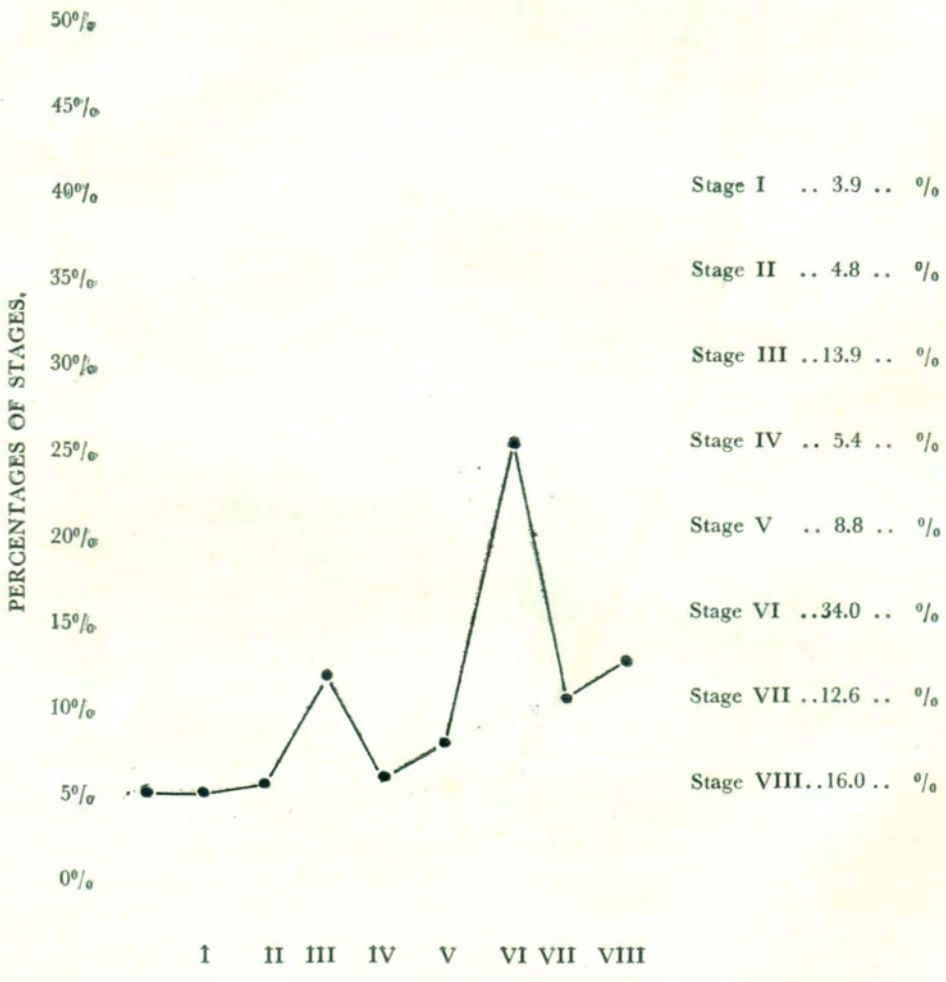
QUANTITATIVE STUDIES OF SPERMATOGENESIS

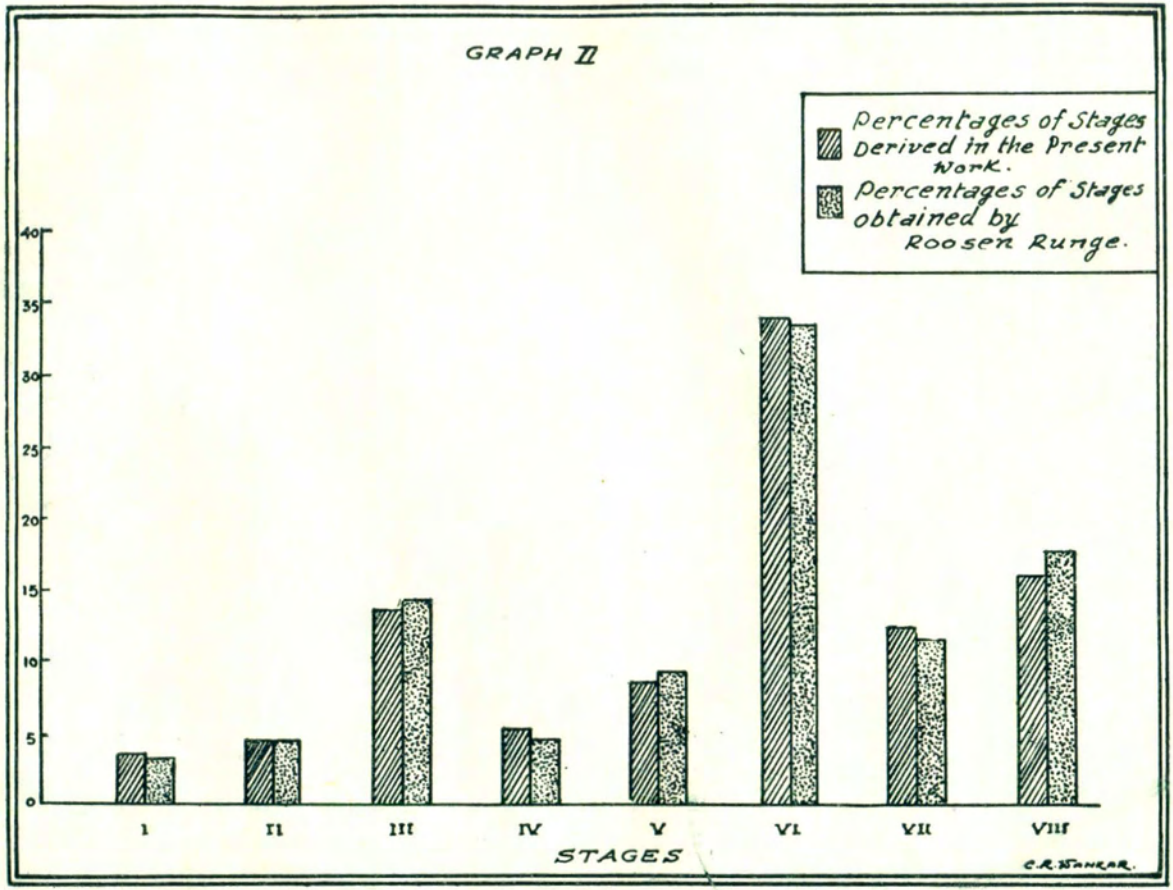
Group No. 1 CONTROL RATS. No. of Rats :—6

Average Size of Testis :—12.0 m.m. × 14.5 m.m. Average Weight of Testis :—0.94 G.

No. of Tubules Counted :—2400 Weight of Testis per 100 G. of Body Wt:—0.53 G.

GRAPH No. I.





COMPARISON OF THE PERCENTAGES OF STAGES OF SPERMATOGENESIS

DISCUSSION

"Periodic acid Fuchsin-Sulfurous acid technique" (Leblond and Clermont, 1952) used in the present investigation differentiates the various cellular transformation occurring during spermatogenesis in a distinct and a precise manner. Iron haematoxylin staining used by Roosen Runge (1950) was also tried in the present work, but it did not give as satisfactory results. Preparation stained by haematoxylin-eosin were found to be unsuitable for such work.

Slight variation in the average values for different stages were observed in the present investigation when compared with those of Roosen Runge (Graph II).

Average values of stages—I, IV, VI and VII were higher by 0.2%, 0.6%, 0.4% and 1%, respectively, from that of Roosen Runge while values for stages III, V, and VIII were lower by 0.6%, 0.6% and 1.6%, respectively (Graph II).

The values of the quantitative approach to a problem as compared to the qualitative cannot be overstressed. The introduction of the quantitative analysis to determine the percentages of the stages of the cycle of seminiferous epithelium of the tubule, is therefore, an important advancement in the field. Quantitative study of spermatogenesis is an important method for exploring the various aspects of male sterility and fertility as it can give an early indication of the variation in spermatogenesis. This method should also enable us to predict the earliest changes preceding tubular degeneration and regeneration, and is likely therefore to afford an extensive scope of further useful work.

SUMMARY

Quantitative studies on spermatogenesis based on Roosen Runge's classification and using "Periodic acid Fuchsin sulfurous acid technique" (Leblond & Clermont, 1952) was undertaken in normal healthy albino rats (Kasuali Strain) weighing between 150-200 grams, maintained on the synthetic diet for the period of 30 days.

A total of 2,400 tubular cross-sections in all were studied in the present investigation. An average value of 3.9%; 4.8%; 13.9,% 5.4%; 8.8%; 34.0%; 12.6% and 16.0% were observed respectively for the stages I to VIII. These values corresponded more or less with those reported by Roosen Runge, (1950).

The value of this quantitative approach in the study of earliest changes preceding tubular degeneration and regeneration has been stressed.

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