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A Quantitative Study of Primordial Germ Cells in the Male Rat

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WITH FOUR PLATES

INTRODUCTION

IN mammals, as in other vertebrates, primordial germ cells arise extra-gonadally and migrate to the genital ridges (see Franchi, Mandl & Zuckerman, 1962). In the rat, the gonads undergo sex differentiation on the 14th day of gestation, i.e. some 2 days after the arrival of the primordial germ cells.

In the female rat, the number of oögonia increases sharply, due to active mitotic proliferation, between the 14th and 18th day of gestation. Thereafter, the majority of germ cells enter the prophase of meiosis, and thus, by definition, become oöcytes. A large number of oöcytes undergo spontaneous degeneration, with the result that the total population decreases from a peak of about 75,000 (at 18·5 days) to about a third that number 2 days after birth. The decrease in the population is due to three distinct 'waves' of degeneration occurring at specific developmental stages (Beaumont & Mandl, 1962). The degenerating oöcytes are removed from the ovary by the phagocytic activity of neighbouring somatic cells (Franchi & Mandl, 1962).

Corresponding quantitative studies on the male foetal rat are incomplete. Beaumont's (1960) observations suggest that mitotic activity of primordial germ cells ceases at about the same stage of intra-uterine life in the two sexes. Clermont & Perey (1957) reported that the number of primordial germ cells seen in cross-sections of the seminiferous cords decreases sharply between late foetal life and 4 days *post partum* (*p.p.*), when many of those which survive divide mitotically to give rise to the first generation of spermatogonia type-A (the 'stem-cells' of future spermatogenetic cycles; Leblond & Clermont, 1952; Clermont & Leblond, 1953; Clermont, 1962). Since the counts made by Clermont & Perey were restricted to establishing numbers of cells seen in circular

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cross-sections of seminiferous cords, whose lengths increase with age (Clermont & Huckins, 1961), it is not possible to use their data for the calculation of the absolute numbers of germ cells.

Clermont & Perey (1957) also noted that large numbers of germ cells undergo degeneration during the first few days after birth, and 'disappear'. The process of degeneration is characterized by a sharp increase in the size of the nucleus, fragmentation of the nuclear membrane, and finally dissolution of the nucleus. Similar qualitative observations have been made by Beaumont (1960), Starkie (1961), Harding (1961) and Hughes (1962). Since the degenerating germ cells are not immediately compensated for by mitotic activity of normal cells, it would appear likely that the total population of germ cells temporarily decreases until such time as it is augmented again by the formation of spermatogonia type-A.

In a further quantitative study, Roosen-Runge & Anderson (1959) estimated the total volume of testicular tissue occupied by primordial germ cells at different ages before and shortly after birth. They did not, however, correlate these with either measurements of individual germ cells at different ages (from which the total population could have been calculated), or with differential counts of normal and degenerating germ cells. Nonetheless, their results clearly show that the volume of 'germinal tissue' rises from the 15th to the 19th day of gestation, apparently due to the mitotic activity of germ cells, whereafter it decreases first slowly, and later rapidly. The decrease is presumably associated with degeneration of an unknown proportion of the germ cells; but the actual extent to which the population is depleted cannot be determined in the absence of cell measurements.

The present study was therefore undertaken in order to estimate the absolute numbers of germ cells in the male foetal and neonatal rat, and to compare the results with those already obtained for the female. In the first instance, the most mature animals studied were aged 4–4·5 days *p.p.* However, the results of this initial quantitative study, together with observations made by electron microscopy (Franchi & Mandl, unpublished observations), suggested that the investigation should be extended to animals aged between 5 and 6·5 days *p.p.*, thus including the first stages in the differentiation of spermatogonia type-A.

MATERIALS AND METHODS

Animals

Forty-five adult female rats of the Birmingham colony were housed with males of proven fertility, and the time of mating estimated as before (see Beaumont & Mandl, 1962). Twenty-seven were killed at different stages of gestation, ranging from 14·5–21·5 days *post coitum* (*p.c.*). The remaining eighteen were allowed to give birth to litters; neonatal males were killed at different intervals between a few hours and 6·5 days after birth.

A second group of thirteen female rats was kept under reversed lighting conditions (darkness: 4 a.m.–4 p.m.; light: 4 p.m.–4 a.m.), and killed between 17 and 21 days *p.c.* (see Mandl, 1963).

The testes of 111 foetal and neonatal specimens were used for the quantitative study (see Table 1).

Autopsy

The does were killed by means of chloroform vapour. The foetuses were rapidly dissected out and the testes removed. In the case of early foetal specimens, the caudal half of the whole embryo was fixed (see Beaumont & Mandl, 1962) and the gonads sexed histologically. Neonatal rats were killed by decapitation. The testes were fixed in Bouin's aqueous fluid, the fixation time varying according to the size of the specimen (*ca.* 2–24 hr.).

Histological procedures

The testes were cut serially at 5μ and stained with Weigert's iron haematoxylin; in some cases, they were lightly counterstained with 'chromotrop 2R'.

Quantitative histological studies

Two groups of animals were used. Ninety-three specimens, whose ages ranged from 14·5 days *p.c.* to 4·5 days *p.p.*, are included in group A. Group B comprises eighteen animals aged 4–6·5 days *p.p.*

In view of the difficulties inherent in making a quantitative assessment of the population of germ cells in the developing testis (see below), three different methods have had to be devised. Methods I and II were used exclusively for animals in group A. Method III was employed for all animals in group B, and also for the eldest in group A (aged 3–4·5 days *p.p.*). The latter animals provide a link between the two groups, and thus permit a comparison of the results obtained by the different methods.

Determination of testicular volumes

The volume of the testes was measured planimetrically from drawings of the outline of the organ at magnifications ranging from $\times 22 \cdot 7$ to $\times 108$, depending on the age of the specimen. Every 10th section was projected and drawn for specimens aged 14·5–21 days *p.c.* inclusive, and every 20th for older specimens in group A. The sample was reduced to every 40th section for animals in group B.

In order to determine the variability between the two gonads of the same individual, the two testes were measured separately for sixty-seven specimens. The first testis to appear in serial sections was designated *a*, and the second *b*; thus right and left testes were probably distributed randomly between these two groups.

Measurements of individual germ cells

Group A. The size of resting germ cells at different stages of development was measured by means of a camera lucida at a magnification of $\times 2700$. Sixty cells, considered to be histologically normal, were measured for each age group (fifteen from each of four randomly selected specimens), and the volumes estimated as before (see Beaumont & Mandl, 1962). The size of dividing primordial germ cells was measured in five groups of animals aged 14·5–17·5 days *p.c.* Due to the very low incidence of dividing cells in specimens aged 18 and 18·5 days *p.c.*, their mean volume was calculated by extrapolating the regression lines (see below).

Measurements were also made for the nuclei of the resting germ cells at all the developmental stages, and for spermatogonia type-A in animals aged 4–4·5 days *p.p.*

Regression analyses were made according to the formula $y = a + bx$, where y = age in days *p.c.*

Group B. The nuclear volume of gonocytes in animals aged 4–6·5 days *p.p.* was determined from measurements of thirty cells (fifteen normal and fifteen degenerating) for two specimens in each age group.

Estimation of number of germ cells

Method I. The number of germ cells in the testes was calculated according to the method outlined by Beaumont & Mandl (1962) for oögonia and oöcytes.

The relative volume of testicular tissue occupied by germ cells ('germinal tissue') was assessed by Chalkley's (1943) method. Sections were selected at random, and scanned under an oil immersion objective. An average of 750 'hits' in each specimen were scored and classified into the following groups: resting primordial germ cells; dividing primordial germ cells; 'supporting' cells (to which the apparent 'lumen' within the seminiferous cords was added; see below) and interstitial cells. A total of 74,415 'hits' was scored for the ninety-three specimens in group A.

The population of germ cells was determined by dividing the volume of 'germinal tissue' by the calculated mean volume of germ cells at the corresponding stage of development (see Beaumont & Mandl, 1962).

Method II. Due to the difficulty of differentiating the apparent 'lumen' of the seminiferous cords from the cytoplasm of germ cells, a further study was made on the fifty-five specimens aged 19 days *p.c.* to 4·5 days *p.p.*

Using Chalkley's method again, 'hits' *within* the seminiferous cords were classified as germinal cytoplasm or 'lumen' (pooled), nuclei of primordial germ cells, and—in the oldest specimens—nuclei of spermatogonia type-A. Four hundred or more 'hits' were scored for each specimen (total 25,761).

The volume of tissue within the seminiferous cords, determined by Method I, was divided by the percentage 'hits' scored by the two types of nuclei. The resulting value was then divided by the calculated mean *nuclear* volume of germ cells at the corresponding stage of development.

Method III. Due to the relatively greater increase in the volume of non-germinal (cf. germinal) tissue during the first week of post-natal life, sampling errors involved in the use of Chalkley's method are liable to be high. A third method for assessing the population of germ cells in older specimens was therefore called for.

The percentage of 'tubular' tissue within the testis was estimated by Chalkley's method. The diameter of twenty seminiferous tubules (from the basement membrane), cut at right angles to their long axis, was measured for each animal by means of a camera lucida (magnification ×790). The mean radius was calculated, and values obtained for the area of one circular cross-section, and the volume of tissue occupied by 250 tubular cross-sections cut at 5μ. The calculated total volume of tubular tissue, divided by the volume occupied by 250 cross-sections of tubules, was subsequently used as a multiplication factor for the estimation of the total number of germ cells (see below).

Germ cells in 250 tubular cross-sections were enumerated and classified into the following groups: normal, lysing and dividing gonocytes; 'transitional' cells; and spermatogonia type-A. These cells were distinguished by their nuclear characteristics (see below). As the cells counted lacked a clear-cut small 'marker' (e.g. a single nucleolus), correction factors for each cell-type were determined. Forty primordial germ cells, and twenty cells classified as dividing, 'transitional' and spermatogonia type-A, were traced serially and the number of sections in which their nucleus was visible was counted. Only cells which were easily identifiable by virtue of their topographical position were used in this analysis. The correction factors were as follows: rats aged 3–3·5 days *p.p.*: resting gonocytes, 2·12; rats aged 4–6·5 days *p.p.*: resting gonocytes, 2·25; dividing gonocytes, 2·15; 'transitional' cells, 2·05; spermatogonia type-A, 1·50.

The population of germ cells was then calculated for each specimen by multiplying the counts/250 tubular cross-sections by the appropriate multiplication factor, and dividing by the correction factor. The following example will illustrate the procedures used:

Total volume of testes	4·0 mm.^{3}
Per cent. tubular tissue (Chalkley's method)	75
Calculated volume of tubular tissue	3·0 mm.^{3}
Mean diameter of seminiferous tubules	80μ
Mean area of cross-section (πr^2)	5072μ
Volume occupied by 250 cross-sections cut at 5μ	$6\cdot284\times10^6\ \mu^3$
Multiplication factor: $\dfrac{3\times10^9}{6\cdot284\times10^6}$	477·4
Correction factor for resting gonocytes	2·1

Number of resting gonocytes counted in 250 cross-sections	300
Total population of germ cells: $\dfrac{300 \times 477 \cdot 4}{2 \cdot 1}$	68,200

Corresponding estimates were made for dividing gonocytes, 'transitional' cells and spermatogonia type-A, where present.

The total length (h) of seminiferous tubules in the testis was calculated by dividing the estimated volume of tubular tissue ($\pi r^2 h$) by the area of seminiferous tubules cut at right angles (πr^2).

Determination of mitotic index of germ cells

Numbers of germinal mitoses were estimated, for specimens aged 14·5–18 days *p.c.*, from counts made on every 20th serial section (cf. Beaumont & Mandl, 1962). An approximate mitotic index was calculated by dividing the number of germinal mitoses by the total calculated population of germ cells.

Determination of volume of non-germinal elements

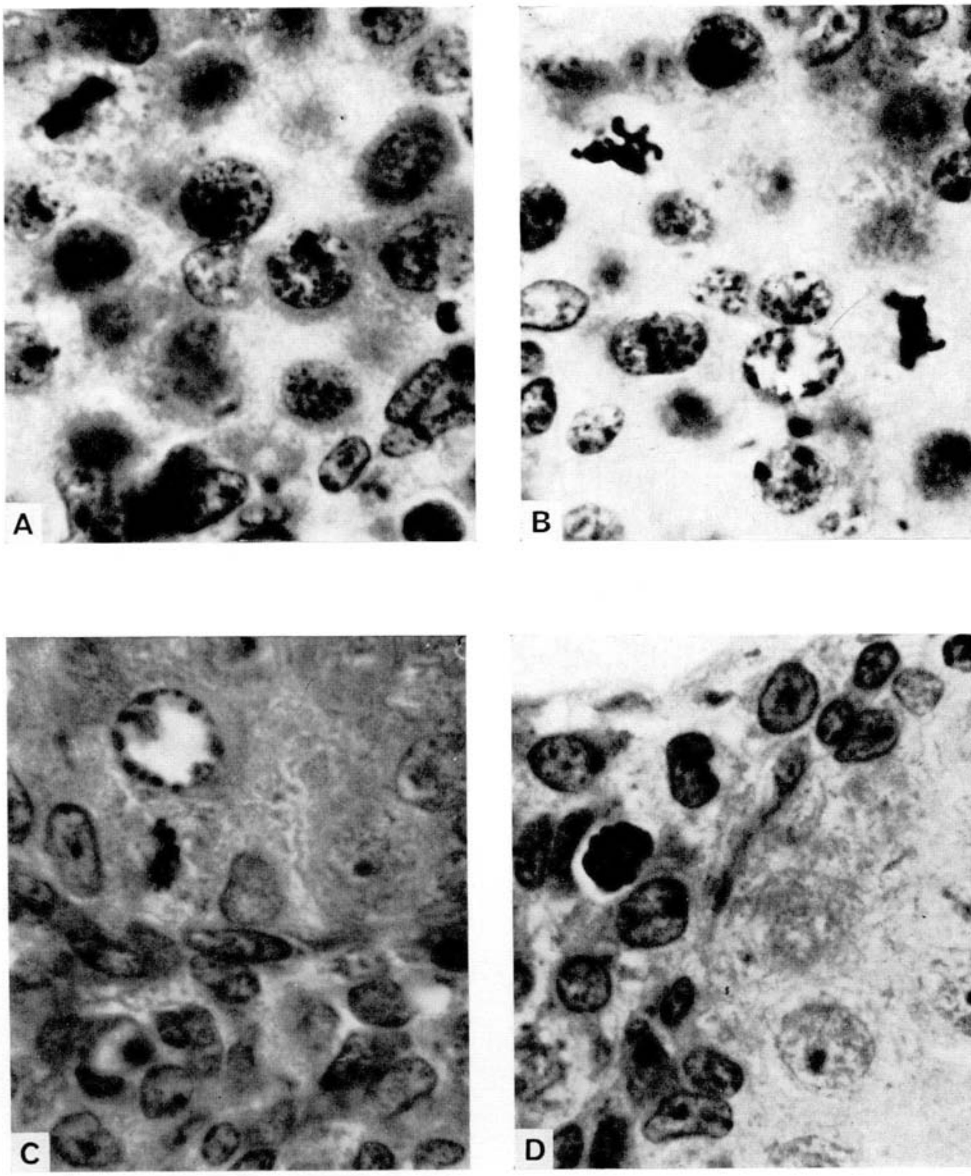
The volume of tissue occupied by (i) 'supporting' cells (including the 'lumen' of the seminiferous cords), and (ii) interstitial cells was calculated from the percentage 'hits' scored by these elements in the initial analysis (group A, Method I). No attempt was made to differentiate interstitial cells in specimens aged less than 18 days *p.c.* stained by the routine procedure. 'Supporting' cells could be differentiated earlier (16·5 days *p.c.*) but measurements were not made for specimens aged less than 17·5 days.

RESULTS

Qualitative observations

The testis first becomes distinguishable from the ovary at 14·5 days *p.c.* by the presence, beneath the germinal epithelium, of a distinct tunica albuginea overlying prominent blood vessels. The primordial germ cells or gonocytes, which are larger than the somatic cells and possess spherical or nearly spherical nuclei (Plate 1, fig. A), are arranged in cords. The latter are the precursors of the seminiferous tubules and later become canalized. In contrast, the ovary lacks a tunica albuginea, and the germ cells (oögonia) are distributed more or less evenly throughout the section. The histological appearance of the germ cells themselves, however, is essentially similar in the two sexes until about the 17th day of gestation.

Between 14·5 and 18·5 days *p.c.* mitoses of germinal cells (Plate 1, figs. A–C), recognized by virtue of their large size (cf. somatic mitoses), are relatively common. The gonocytes increase in size as development proceeds (Plates 1



EXPLANATION OF PLATES

Histological preparations (5 μ), stained with Weigert's iron haematoxylin. Magnification × 1250 (Plates 1 and 2) and 2100 (Plates 3 and 4).

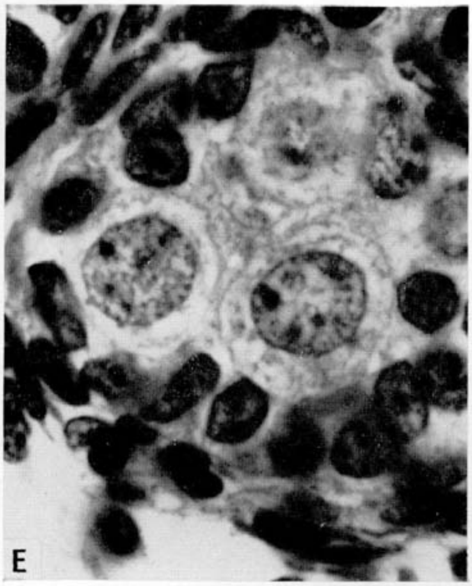
PLATE 1

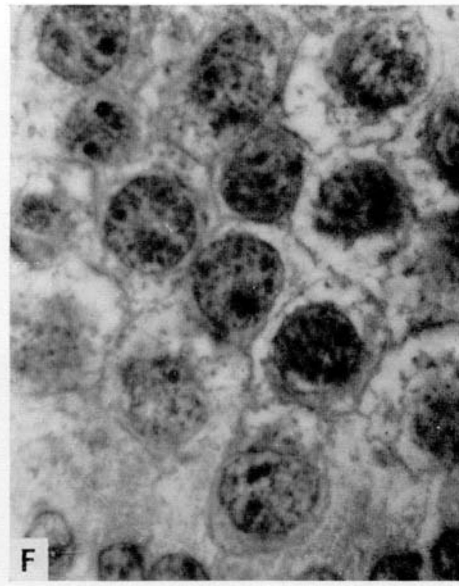
FIG. A. Normal primordial germ cells (15·5 days *p.c.*).

FIG. B. Primordial germ cells at the prophase and metaphase of mitosis (15·5 days *p.c.*).

FIG. C. Primordial germ cells at the prophase and metaphase of mitosis (17·5 days *p.c.*).

FIG. D. Above: interstitial cell at metaphase of mitosis; below: normal primordial germ cell at interphase (18·5 days *p.c.*).





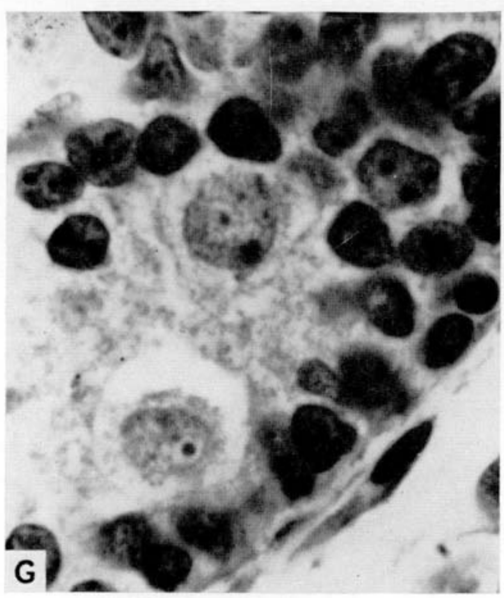


PLATE 2

FIG. E. Normal primordial germ cells at interphase (21·5 days *p.c.*).

FIG. F. Primordial germ cells at interphase. A number show changes previously believed to indicate degeneration, e.g. breakdown of nuclear membrane and spreading of nuclear contents (20·5 days *p.c.*).

FIG. G. Primordial germ cells showing similar 'degenerative' changes (1–1·5 days *p.p.*).

and 2, cf. figs. A, D, E). A few germ cells which are not incorporated into the seminiferous cords, and appear in the interstitial spaces, show signs of degeneration. The Leydig cells become distinguishable from other somatic components by their rounded nuclei, large cytoplasmic/nuclear ratio, cytoplasmic eosinophilia and topographical distribution (see Roosen-Runge & Anderson, 1959). Mitotic divisions of Leydig cells are commonly seen (Plate 1, fig. D).

Mitoses of germ cells are rarely seen in specimens aged 18·5 days *p.c.* to 3·5 days *p.p.* The appearance of the gonocytes changes as, with the increase in their size, the nucleus becomes less chromophil, the nucleoli round and prominent, and the cytoplasmic/nuclear ratio rises (Plate 2, figs. E, F; cf. Plate 1, fig. A). In specimens aged 18·5–20·5 days *p.c.* it was noted that a proportion of the germ cells are somewhat indistinct in outline, possibly due to a temporarily reduced affinity for stain. From the 20th day of gestation onwards, large numbers of germ cells show changes which have hitherto been considered indicative of degeneration (see e.g. Clermont & Perey, 1957). Such cells are characterized by an enlargement of the nucleus; crinkling and/or fragmentation of the nuclear membrane; and contraction of the cytoplasm, resulting in the formation of clear 'spaces' (Plate 2, figs. F, G). The results of the quantitative study (see below), together with recent observations on the ultrastructure of developing testes (Franchi & Mandl, *unpublished*) suggest, however, that degeneration does not set in until at least 2 days after birth. The electron microscopic studies have also confirmed the conclusions, based on light microscopy (e.g. Clermont & Perey, 1957; Sapsford, 1963), that the apparent 'lumen' seen within the seminiferous cords from about 20 days *p.c.* onwards (cf. Plate 2, figs. F, G) effectively consists of cytoplasmic extensions of the 'supporting' cells. A *true* lumen appears no earlier than about a fortnight after birth (Sapsford, 1963).

As judged by both light and electron microscopy, testes of rats aged 4–6·5 days contain several morphologically distinguishable cell-types. Many primordial germ cells appear normal (Plate 3, fig. H). Others almost certainly undergo lysis at this time. The degenerative changes observed include complete breakdown of the nuclear membrane and widespread dispersal and dissolution of the nuclear contents (Plate 3, figs. I, J). A further proportion of the gonocytes appear to be undergoing differentiation and migrate towards the basement membrane of the seminiferous cord. Such cells lose their rounded outline and become elongated (Plate 3, fig. K); their nucleus is frequently lobed (Plate 3, fig. L). The nucleoli become associated with the nuclear membrane and are rod-shaped, whilst the chromatin becomes increasingly condensed and chromophilic. Since these cells appear to represent a stage in the transformation of primordial germ cells into spermatogonia, they are designated 'transitional' cells. They first appear at 4 days *p.p.* and increase in frequency up to about 6 days.

The differentiation of primordial germ cells is associated with a renewal of mitotic activity (Plate 4, figs. M, N); the incidence of dividing 'supporting' cells is also fairly high at this time.

Spermatogonia type-A are first found in appreciable numbers at 5–5·5 days *p.p.* Their nuclei are smaller than those of gonocytes or 'transitional' cells; they are ovoid in shape and possess a densely staining chromatin with several prominent nucleoli. These cells are characteristically aligned on the basement membrane of the seminiferous cord (Plate 4, fig. O).

The testes of three of the ten animals aged 4–4·5 days in group A contained spermatogonia type-A, as well as occasional germ cells undergoing mitosis. In the remaining seven animals, the renewal of mitotic activity had not as yet set in. In view of this striking difference between these two groups, separate means were calculated for the quantitative observations on the testes (*i* and *ii*; see Tables 1, 2, 3, 5, 6).

Quantitative observations

Testicular volume

The testes grow steadily between 14·5 days *p.c.* and 6·5 days *p.p.* (Table 1). Separate measurements of the two gonads (a and b) in the same individual showed that the variability between them is negligible ($a/b = 100\cdot39 \pm 1\cdot24$). It seemed justifiable, therefore, to base the estimates of testicular volume for the remaining forty-four animals on measurements of a single gonad (multiplied by two).

The size of germ cells

The germ cells increase in size progressively with age (see Table 3). Since the growth of the nucleus is less rapid than that of the entire cell, the ratio of nucleus:cytoplasm decreases as development proceeds (Text-fig. 1).

Regression analyses were computed separately for resting gonocytes and their nuclei, and for dividing gonocytes (group A). The slopes of the regression lines were significantly different from zero in all cases, the regression coefficients b being $157\cdot5 \pm 8\cdot71$, $30\cdot05 \pm 1\cdot80$ and $105\cdot17 \pm 39\cdot71$ for the three groups respectively.

Measurements of the nuclear volume of gonocytes in animals aged 4–6·5 days *p.p.* (group B) were not included in the regression analysis. The findings confirm, however, that the increase in the nuclear volume of gonocytes is maintained up to the time of cellular differentiation and/or renewal of mitotic activity (see below). Although qualitative observations suggest that degenerating gonocytes undergo both nuclear and cytoplasmic swelling, measurements of normal and degenerating nuclei failed to show any consistent difference between the two:

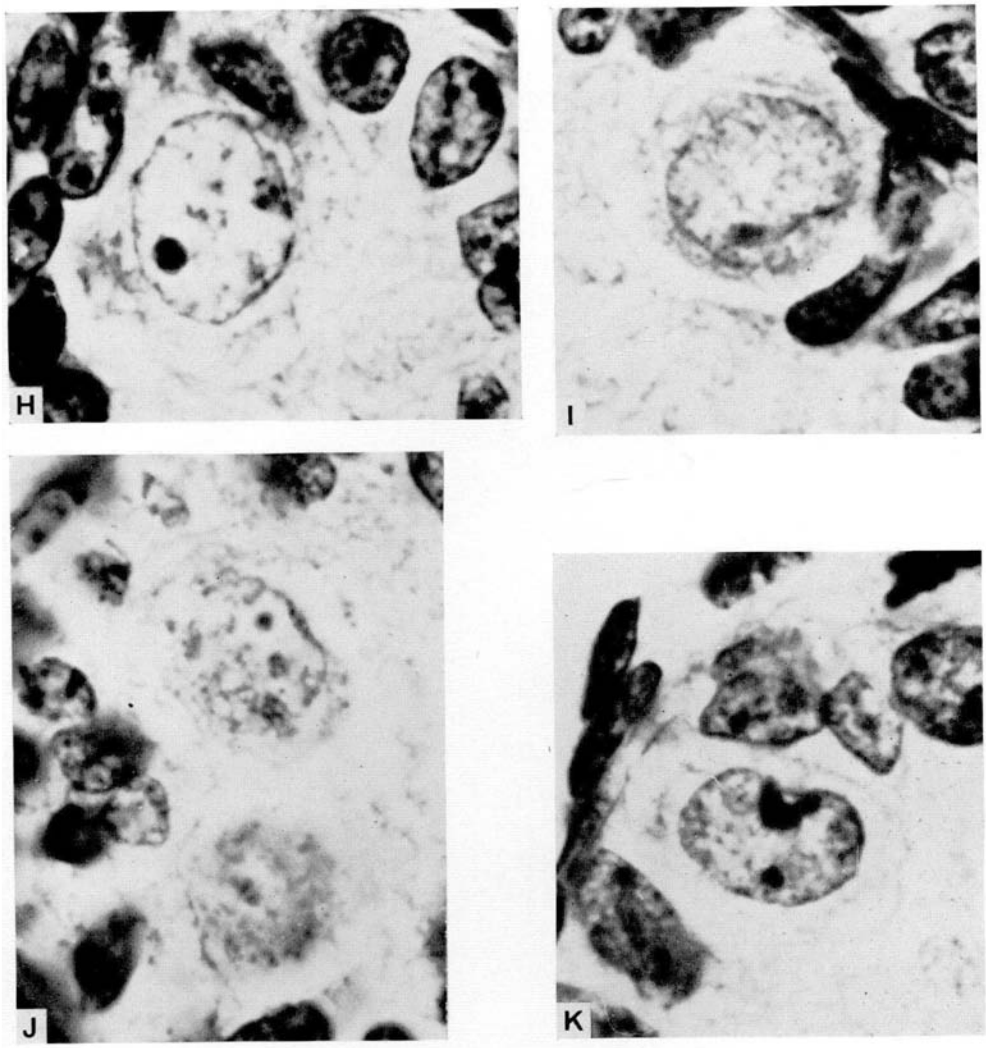


PLATE 3

FIG. H. Primordial germ cell at interphase (6·5 days *p.p.*).

FIG. I. Primordial germ cell classified as degenerating (6·5 days *p.p.*).

FIG. J. Primordial germ cells showing complete disintegration of the nucleus (6·5 days *p.p.*).

FIG. K. 'Transitional' cell in contact with the basement membrane (6·5 days *p.p.*).

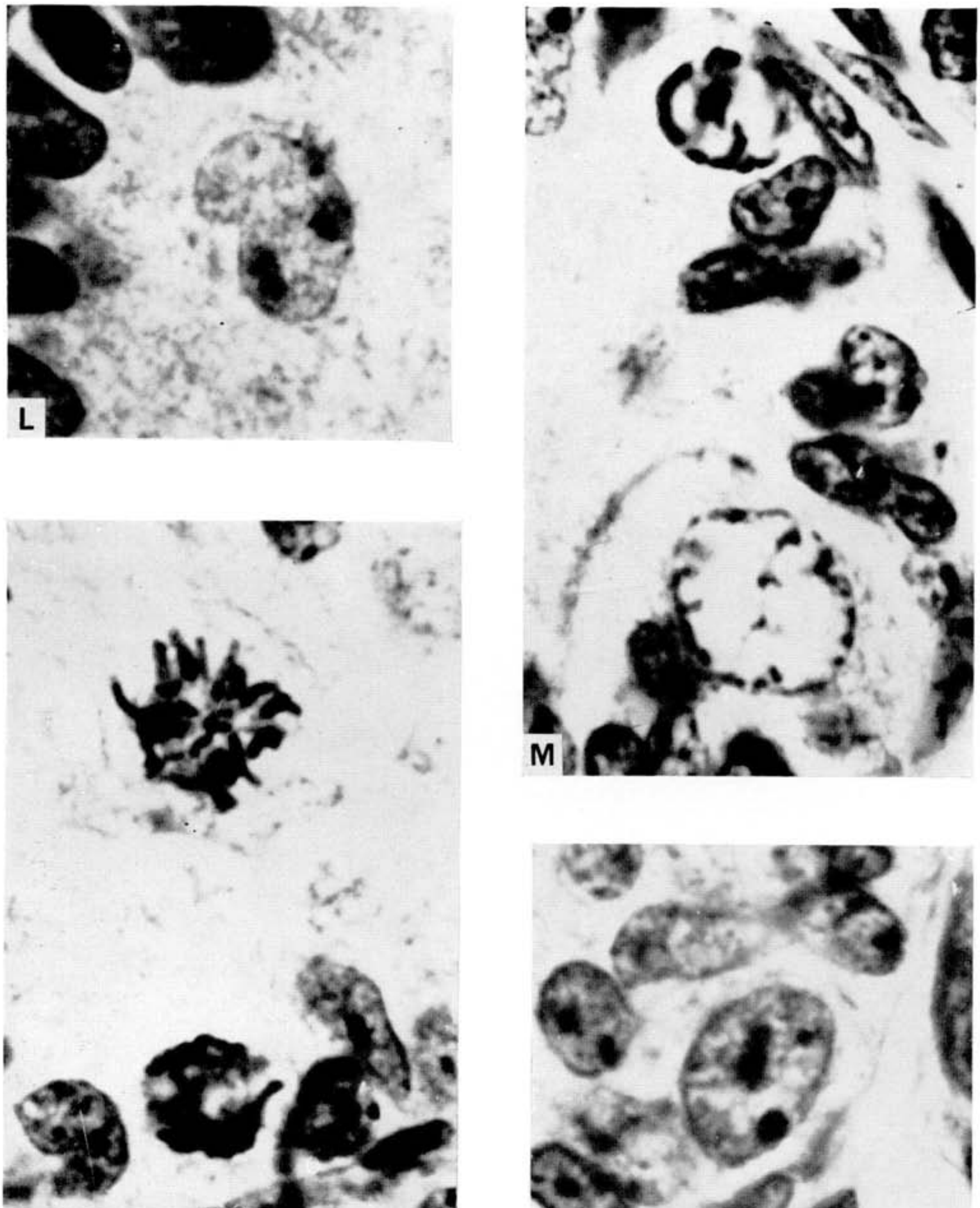


PLATE 4

FIG. L. 'Transitional' cell with lobed nucleus (6 days *p.p.*).
FIG. M. Germinal and somatic cell at prophase of mitosis (5 days *p.p.*).
FIG. N. Germinal and somatic cell at metaphase of mitosis (6 days *p.p.*).
FIG. O. Spermatogonium type-A (6·5 days *p.p.*).

Age (days p.p.)	*Volume of normal nuclei* (μ^3)	*Volume of 'degenerating' nuclei* (μ^3)
4	467	493
4·5	546	465
5	608	530
5·5	602	524
6	646	678
6·5	589	646

It should be noted, however, that it is extremely difficult to make accurate drawings of degenerating nuclei under the camera lucida. First, the recognition of the onset of degeneration is difficult; and second, since the nuclear membrane frequently breaks down, measurements of nuclear size are apt to be unreliable. Furthermore, the breakdown of the nuclear membrane is likely to hinder the recognition of cells in advanced stages of lysis.

TABLE 1

Mean testicular volume in foetal and neonatal rats

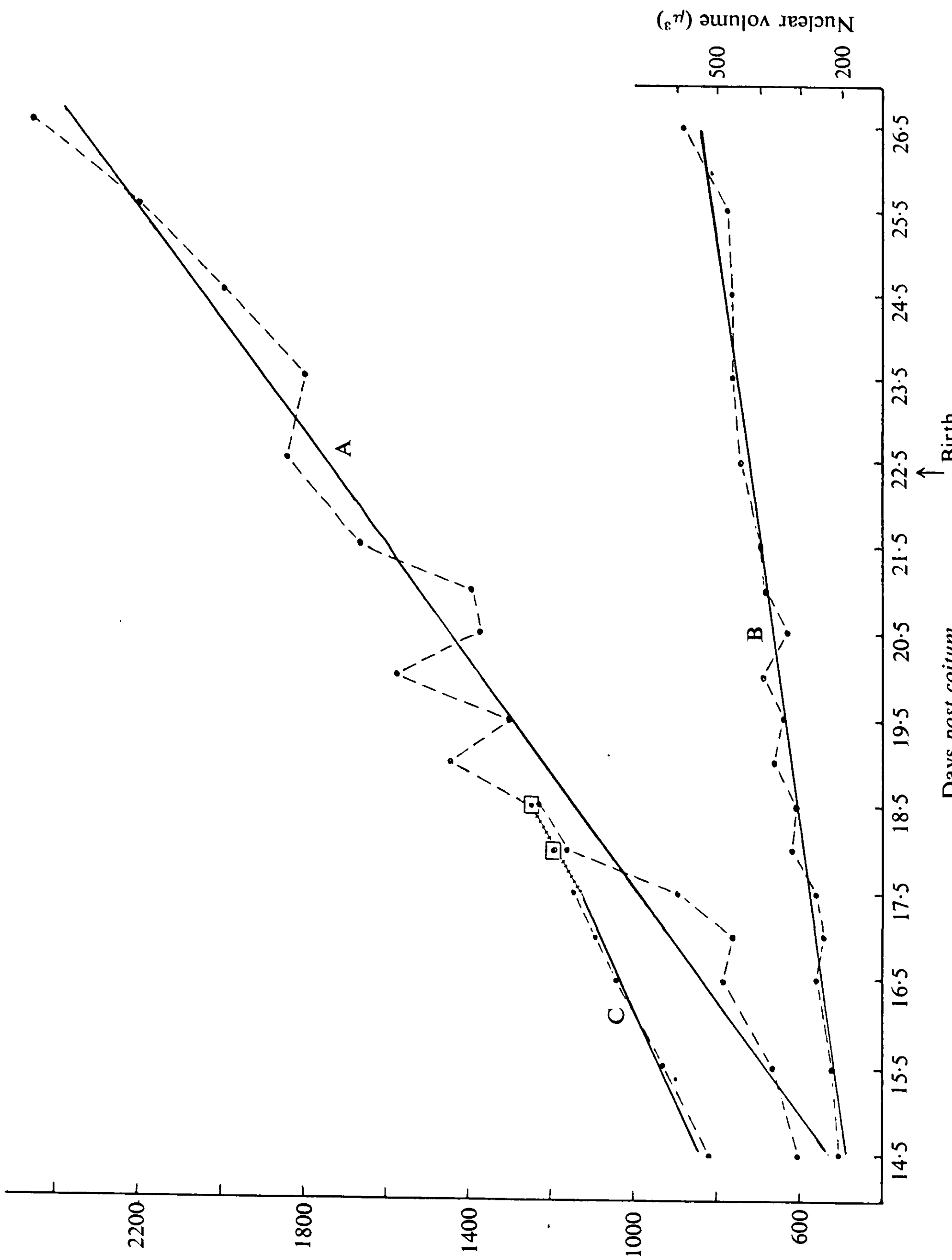
	Age (days)	*No. rats*	*Mean volume* (mm.$^3 \times 10^{-3}$)
Group A	14·5 *p.c.*	6	52·8 ± 5·1
	15·5 *p.c.*	7	117·1 ± 5·7
	16·5 *p.c.*	4	201·0 ± 16·4
	17·0 *p.c.*	4	266·2 ± 29·2
	17·5 *p.c.*	5	345·2 ± 13·7
	18·0 *p.c.*	6	526·9 ± 45·4
	18·5 *p.c.*	6	593·1 ± 30·0
	19·0 *p.c.*	3	738·4 ± 20·4
	19·5 *p.c.*	4	891·6 ± 59·5
	20·0 *p.c.*	5	1243·2 ± 16·5
	20·5 *p.c.*	4	1111·1 ± 100·7
	21·0 *p.c.*	7	1395·2 ± 60·7
	21·5 *p.c.*	5	1842·6 ± 165·9
	Neonatal	4	2436·0 ± 205·0
	1–1·5 *p.p.*	4	3118·7 ± 307·6
	2–2·5 *p.p.*	4	4159·9 ± 46·5
	3–3·5 *p.p.*	5	4835·2 ± 171·2
	4–4·5 *p.p.* (*i*)	7	6082·2 ± 342·1
	(*ii*)	3	8965·3 ± 356·4
Group B	4	3	6544 (5685–7740)
	4·5	3	5762 (5322–6471)
	5	3	6355 (6128–6597)
	5·5	3	6597 (5443–8004)
	6	3	8829 (8229–9800)
	6·5	3	8675 (7751–10430)

p.c. = *post coitum*; *p.p.* = *post partum*.

Due to the small number of specimens in each age-group in B, standard errors of the mean are replaced by the ranges.

i = animals aged 4–4·5 days *p.p.*, possessing few or no spermatogonia type-A.

ii = animals aged 4–4·5 days *p.p.*, in whose testes numerous spermatogonia type-A were already present.



TEXT-FIG. 1. Measurements and regression lines calculated for the growth of: A, resting primordial germ cells; B, their nuclei; and C, dividing primordial germ cells, at different stages of late foetal and early neonatal life. Two additional values for dividing cells, obtained by extrapolation (□) are also given (see text).

TABLE 2

Percentage of cell-types, estimated by Chalkley's (1943) method, in foetal and neonatal testes (Group A)

Age (days)	*Total no. cells 'scored'*	*Resting germ cells*		*Dividing germ cells*		*'Lumen' in sem. cords†*		*'Supporting' cells*		*Interstitial cells*	
		Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*
14·5 *p.c.*	3410	18·9	0·58	1·7	0·17	—	—	—	—	—	—
15·5 *p.c.*	4986	23·4	0·95	3·1	0·29	—	—	—	—	—	—
16·5 *p.c.*	3039	27·5	1·76	3·0	0·39	—	—	—	—	—	—
17·0 *p.c.*	3378	26·7	1·44	0·7	0·52	—	—	15·0	1·94	—	—
17·5 *p.c.*	4008	27·6	0·39	0·3	0·12	—	—	15·7	0·97	—	—
18·0 *p.c.*	5031	25·1	0·18	0·1	0·07	—	—	15·4	0·69	9·2	0·58
18·5 *p.c.*	4828	23·0	0·95	0·1	0·04	—	—	15·5	0·82	8·1	0·41
19·0 *p.c.*	2530	21·4	1·00	—	—	—	—	14·6	1·02	7·9	0·71
19·5 *p.c.*	3256	19·3	1·43	—	—	—	—	17·3	1·37	8·5	0·63
20·0 *p.c.*	3738	*16·1	0·26	—	—	—	—	17·2	0·56	7·5	0·58
20·5 *p.c.*	2793	*16·3	0·26	—	—	—	—	17·3	0·60	7·3	0·39
21·0 *p.c.*	5799	11·0	0·44	—	—	6·0	0·85	19·3	0·95	5·8	0·45
21·5 *p.c.*	4290	11·5	0·47	—	—	7·1	0·84	20·2	1·20	6·1	0·72
Neonatal	3244	7·6	0·73	—	—	7·4	0·33	22·3	1·25	4·3	0·67
1–1·5 *p.p.*	3582	7·5	0·49	—	—	8·8	1·07	27·3	0·91	3·5	0·72
2–2·5 *p.p.*	3572	6·4	0·67	0·1	0·05	11·2	0·75	30·6	1·26	4·1	0·54
3–3·5 *p.p.*	4392	5·4	0·55	0·4	0·19	12·6	0·71	30·2	0·45	3·0	0·31
4–4·5 *p.p.* (*i*)	5985	2·7	0·14	0·1	0·05	13·7	1·39	30·3	1·10	2·5	0·26
(*ii*)	2554	5·9	0·54	0·6	0·07	14·4	0·36	36·9	1·32	2·5	0·55

p.c. = *post coitum*; *p.p.* = *post partum*.

i = animals aged 4–4·5 days *p.p.* possessing few or no spermatogonia type-A.

ii = animals aged 4–4·5 days *p.p.* in whose testes numerous spermatogonia type-A were already present.

* Differentiation between germ cells and 'lumen' was difficult at these stages; the values given probably include some 'hits' on tissue outside the indistinct cell membranes of the germ cells.

† Cytoplasm of 'supporting' cells; see text.

TABLE 3

Calculated volume of 'germinal tissue' and population of germ cells (Method I; Group A)

Age (days)	Volume of 'germinal tissue' ($mm.^3 \times 10^{-3}$) Mean	S.E.	Calculated mean volume (μ^3) of germ cells (regression)	Population of germ cells Mean	S.E.
14·5 *p.c.*	10·86	1·10	530	19,897	2041
15·5 *p.c.*	31·07	1·81	687	43,799	3128
16·5 *p.c.*	60·37	2·37	845	70,140	2769
17·0 *p.c.*	73·78	12·41	924	79,474	13,142
17·5 *p.c.*	96·34	3·84	1003	95,905	3788
18·0 *p.c.*	132·31	10·28	1082	122,233	9499
18·5 *p.c.*	136·91	9·79	1160	118,001	8432
19·0 *p.c.*	157·60	6·89	1239	127,202	5553
19·5 *p.c.*	171·30	14·38	1318	129,966	10,914
20·0 *p.c.*	199·90	3·67	1397	143,067	2636
20·5 *p.c.*	180·70	16·61	1476	122,442	11,256
21·0 *p.c.*	153·30	9·15	1555	98,585	5887
21·5 *p.c.*	211·05	16·37	1634	129,159	10,015
Neonatal	182·34	15·52	1752	104,073	8860
1–1·5 *p.p.*	230·42	20·34	1910	120,637	10,653
2–2·5 *p.p.*	268·45	28·76	2067	129,873	13,917
3–3·5 *p.p.*	281·34	28·60	2225	126,444	12,814
4–4·5 *p.p.* (*i*)	170·88	13·36	2383	71,706	5611
(*ii*)	584·85	8·58	*	*	*

p.c. = *post coitum*; *p.p.* = *post partum*.

* Since the testes of animals aged 4–4·5 days (group ii) contained a mixed population of primordial germ cells and spermatogonia type-A, estimates of the total population of germ cells by Method I could not be provided.

For explanations of (*i*) and (*ii*), see Table 2.

The total population of germ cells

The volume of 'germinal' tissue, estimated by Chalkley's (1943) method (see Table 2), increases steadily between 14·5 and 20 days *p.c.* (Table 3). It then remains more or less constant up to the time of birth, whereafter it rises again slightly. A further marked increase in the volume of 'germinal tissue' was

TABLE 4

Numbers of mitotically active germ cells counted in a sample of every 20th section

Age (days)	No. rats	Estimated mean no. dividing germ cells	Estimated mitotic index (%)
14·5 *p.c.*	6	790	5·3
15·5 *p.c.*	5	2064	4·7
16·5 *p.c.*	4	3125	4·5
17·0 *p.c.*	4	1180	1·5
17·5 *p.c.*	5	1312	1·4
18·0 *p.c.*	5	252	0·2

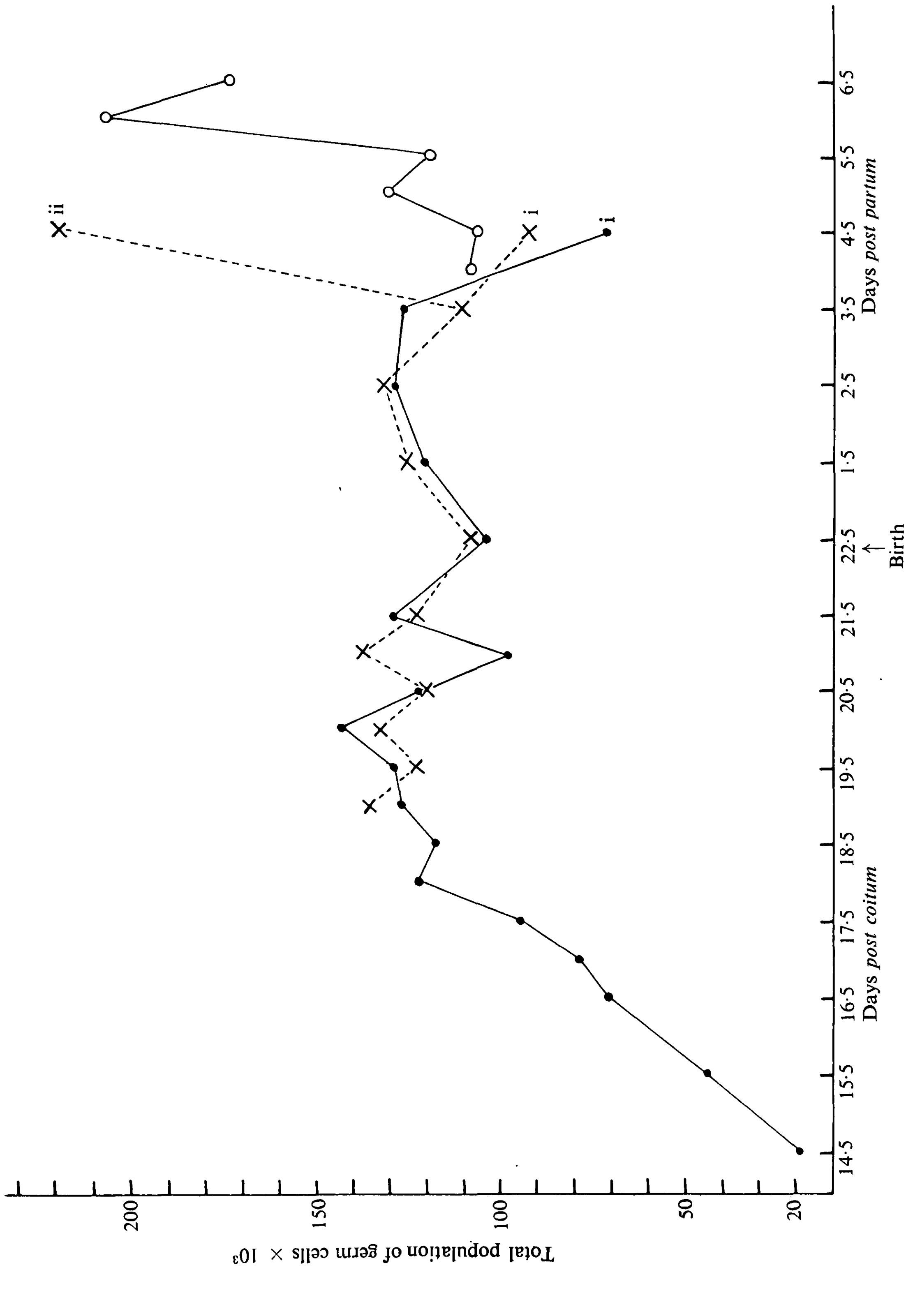
p.c. = *post coitum*.

TABLE 5

Calculated population of primordial germ cells and spermatogonia type-A in late foetal and neonatal rats (Method II; Group A)

Age (days)	*No. cells scored*	*Per cent. germinal nuclei, estimated by Chalkley's method, within seminiferous cords*		*Calculated population of germ cells*			
						Total	
		Primordial germ cells	*Spermatogonia type-A*	*Primordial germ cells*	*Spermatogonia type-A*	*Mean*	*S.E.*
19·0 *p.c.*	1361	28·2	—	135,467	—	135,467	7778
19·5 *p.c.*	1764	24·9	—	123,550	—	123,550	10,080
20·0 *p.c.*	2153	23·8	—	132,799	—	132,799	1897
20·5 *p.c.*	1731	25·1	—	120,664	—	120,664	8539
21·0 *p.c.*	2937	22·4	—	137,688	—	137,688	12,247
21·5 *p.c.*	2136	14·7	—	122,730	—	122,730	9327
Neonatal	2072	12·8	—	108,588	—	108,588	6265
1–1·5 *p.p.*	1853	11·1	—	125,099	—	125,099	18,311
2–2·5 *p.p.*	1879	8·7	—	131,020	—	131,020	6225
3–3·5 *p.p.*	2468	6·3	0·04	108,990	1,068	110,062	10,583
4–4·5 *p.p.* (*i*)	3856	5·1	0·03	91,868	767	92,635	6084
(*ii*)	1551	2·6	2·6	88,919	130,219	219,139	1957

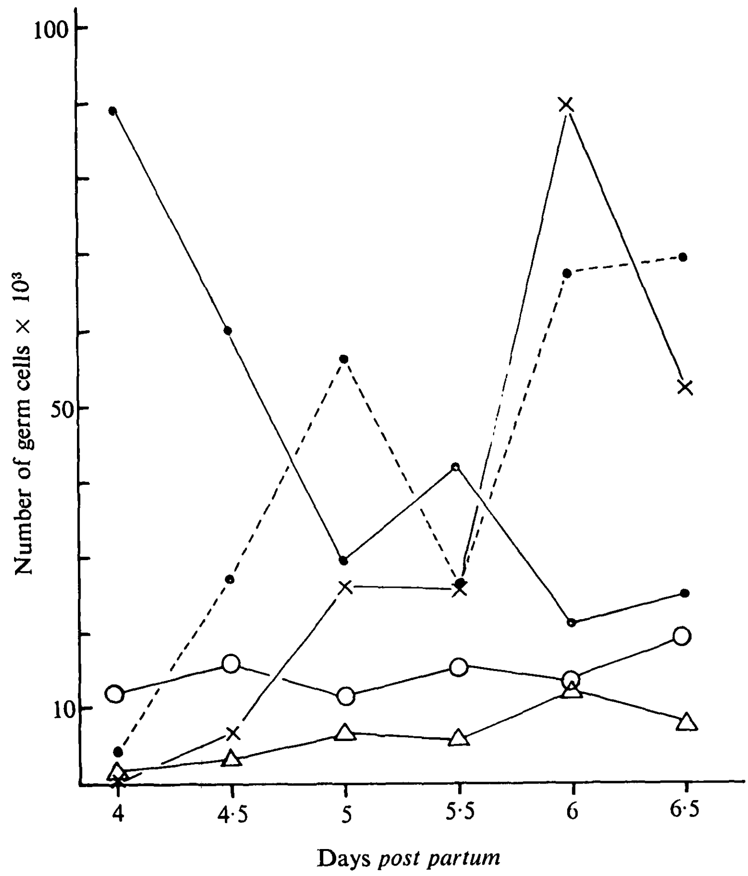
p.c. = *post coitum*; *p.p.* = *post partum*; for (*i*) and (*ii*), see Table 2.



TEXT-FIG. 2. Estimated means for the total population of germ cells at different stages of development. ——●——, Method I; – – × – –, Method II; and ——○——, Method III.

observed in animals, aged 4–4·5 days, whose testes contained spermatogonia type-A (group *ii* in Table 3).

The initial increase in the volume of 'germinal tissue' is clearly correlated with (*a*) mitotic activity, and (*b*) the growth of the primordial germ cells. Estimates of the mitotic index (Table 4) suggest a high rate of division on days 14·5 to 16·5 *p.c.*, which subsequently declines and ceases shortly after 18 days (see also Table 2). A further increase in 'germinal tissue', observed during the first few days after birth, is similarly associated with the renewal of mitotic



TEXT-FIG. 3. Estimated means for the numbers of: (*a*) ——●——, normal gonocytes; (*b*) ——○——, lysing gonocytes; (*c*) ——△——, dividing gonocytes; (*d*) – – ● – –, 'transitional' cells; and (*e*) ——×——, spermatogonia type-A.

activity in primordial germ cells (Table 2), and the appearance of spermatogonia type-A; during this period the primordial germ cells themselves also increase in size.

The total population of primordial germ cells (calculated by Method I) increases steadily from *ca.* 20,000 at 14·5 days *p.c.*, to reach a peak of *ca.* 140,000 at 20 days (Table 3; Text-fig. 2). Estimates by both Methods I and II

TABLE 6

Calculated populations of normal, degenerating and dividing gonocytes, 'transitional' cells and spermatogonia type-A in the testes of neonatal animals (Method III; Groups A and B)

Group	Age (days p.p.)	Gonocytes: Normal resting	Gonocytes: Degenerating	Gonocytes: Dividing	'Transitional' cells	Spermatogonia type-A	Total population of germ cells: Mean	Total population of germ cells: Range	Length of seminiferous cords (mm.) Mean
A	3–3·5	86,060	21,030	—	—	—	107,090	98,240–120,550	1493
	4–4·5 (*i*)	73,730	16,010	1620	1680	—	93,040	81,250–104,940	1765
	(*ii*)	31,360	8940	19,220	82,770	65,840	208,130	191,890–221,570	2651
B	4	87,900	14,670	1520	4380	330	108,800	99,950–124,860	2032
	4·5	60,030	16,670	2950	20,740	6620	107,010	100,440–112,510	1712
	5	29,020	12,520	5920	56,520	26,200	130,180	116,920–141,850	1968
	5·5	44,940	15,060	5350	26,790	26,040	118,180	94,060–155,500	1928
	6	22,450	13,770	13,830	67,130	89,920	207,100	178,030–256,740	2578
	6·5	25,810	19,800	6990	69,890	52,050	174,540	109,000–261,590	2479

For abbreviations, see Table 2.

(Table 5; Text-fig. 2) for specimens aged 20 days *p.c.* to 2–2·5 days *p.p.* are highly variable. Moreover, there appear to be considerable fluctuations between the estimates *within* each age group. It would appear probable, therefore, that the total population of primordial germ cells remains essentially static during the last 2–3 days before, and the first 2 days after birth.

Between 2·5 and 4·5 days *p.p.* there seems to be a slight fall in the total population of germ cells, with the notable exception of animals aged 4–4·5 days (group *ii*) whose testes already contained type-A spermatogonia. These trends are revealed in the results obtained from all three methods of analysis (see Tables 3, 5, 6). The number of germ cells in the testis then increases from less than 110,000 at 4·5 days to *ca.* 200,000 cells at 6·5 days *p.p.* (Table 6).

These changes, which occur in association with the formation of the first generation of spermatogonia type-A, have been studied in greater detail in animals in group B (see Table 6; Text-fig. 3). The population of normal gonocytes decreases from about 90,000 at 4 days to 25,000 at 6·5 days. The number of mitotically active gonocytes rises from about 1500 at 4 days to 7000–14,000 at 6–6·5 days. Estimates of lysing gonocytes indicate that their population remains fairly uniform over the period studied, varying between 10,000 and 20,000. The decline in the number of normal gonocytes is apparently matched by a corresponding rise in the number of cells classified as 'transitional'. The latter precedes the appearance of spermatogonia type-A, which number between 50,000 and 90,000 at 6–6·5 days *p.p.* (Table 6), and thus represent some 30–40 per cent. of the total population of germ cells.

Length of seminiferous cords

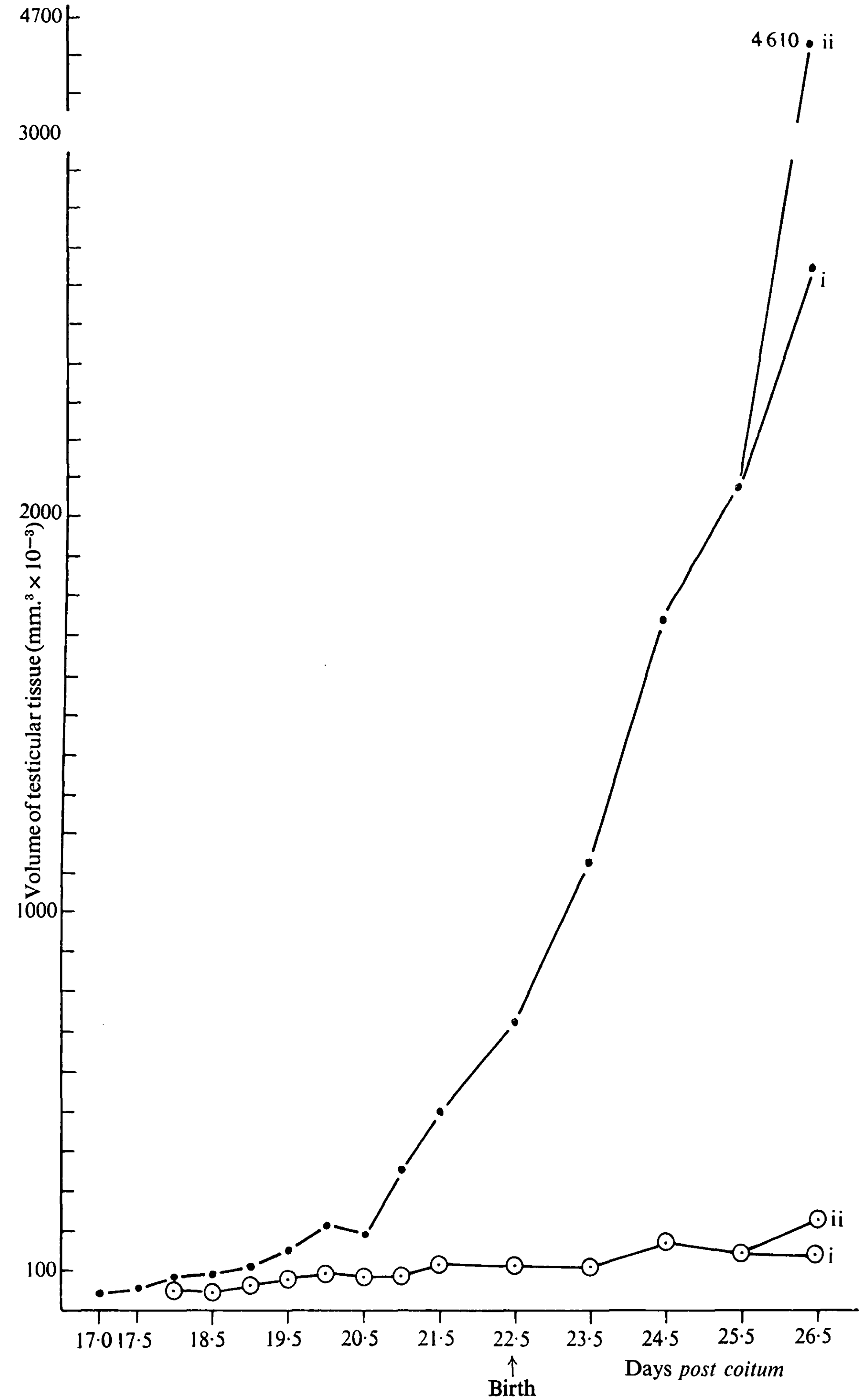
The seminiferous cords increase in length, from approximately 1500 mm. at 3–3·5 days to 2500 at 6–6·5 days *p.p.* (Table 6).

'Supporting' cells

The proportion of the testicular volume occupied by 'supporting' cells increases steadily from 17·5 days *p.c.*, when they first become easily distinguishable from other cells, up to 4–4·5 days *p.p.* (Table 2). The calculated volume of tissue occupied by these cells rises gradually up to the age of 20·5 days *p.c.*; thereafter, the rate of increase is more rapid (Text-fig. 4).

Interstitial cells

Interstitial cells first become histologically recognizable (in sections stained with haematoxylin and 'chromotrop 2R') at the age of 18 days *p.c.* In contrast to the 'supporting' cells, they proliferate relatively slowly, and hence occupy a progressively diminishing proportion of the growing testis (Table 2). There is, however, a small gradual increase in the calculated absolute volume of interstitial tissue as development proceeds (Text-fig. 4).



TEXT-FIG. 4. Calculated volume of testicular tissue occupied by: ——●——, 'supporting' cells, and ——⊙——, interstitial cells.

Validity of methods used

Several sources of error involved in the volumetric estimation of the population of oögonia and oöcytes have already been listed and discussed (see Beaumont & Mandl, 1962). In applying the same technique to the developing testis (Methods I and II), at least as many sources of error have to be taken into account. Indeed, it is probable that due to the relatively faster growth of non-germinal elements in the testis, as compared with the ovary, sampling errors are liable to be greater in the male. In an attempt to overcome this obstacle, the numbers of 'hits' scored by Chalkley's method were considerably higher in the present study (over 700/specimen) than in that on the developing ovary (300 to 400/specimen).

The cell boundary of primordial germ cells in the developing testis is considerably less distinct than that of oöcytes, particularly at late stages of foetal life and shortly after birth. In view of the difficulty of differentiating between the cytoplasm of 'diffuse' gonocytes on the one hand, and that of the 'supporting' cells on the other, the older specimens were subjected to a second analysis (Method II). Although the latter is liable to be more accurate, some difficulty was encountered in differentiating between the nucleoplasm and cytoplasm of some gonocytes, largely due to the fragmentation or faintness of the nuclear membrane. This applied particularly if the pointer lay over a small peripheral section of the nucleus; under these circumstances, differentiation between primordial germ cells and spermatogonia type-A was also somewhat subjective.

TABLE 7

Comparison between estimates of the total population of germ cells by Methods I, II and III

	Calculated population of germ cells		
Age (days p.p.)	*Method I*	*Method II*	*Method III*
3–3·5	126,440	110,060	107,090
	(5)	(5)	(3)
4–4·5 (*i*)	71,706	92,640	93,040
	(7)	(7)	(5)
(*ii*)	—	219,140	208,130
		(3)	(3)

Figures in parentheses indicate number of animals from which means were derived.
For abbreviations, see Table 2.

Although Methods I and II were not entirely independent of one another, the fair agreement between the two estimates (based on cytoplasmic and nuclear size) suggests that the calculated population of germ cells is likely to be at least of the right order (see Text-fig. 2). Moreover, since the counts of 'hits' were made on coded slides (i.e. the observer was unaware of the age of the specimen), the fluctuations in the population reported are unlikely to be due to personal bias. Finally, there is no reason to suppose that the sources of error were significantly higher in estimates for some age groups than for others.

The application of Method III to animals already studied by Methods I and II provides a useful check on the validity of the volumetric method. The results suggest that the observations made on the two groups of animals are strictly comparable. The estimates for total populations of germ cells obtained by Methods II and III show particularly close agreement (see Table 7).

DISCUSSION

The results of the present study show that the number of primordial germ cells or gonocytes in the testis of the rat increases from 14·5 days *p.c.*, when the population is *ca.* 20,000, to reach a peak of some 140,000 at 20 days *p.c.* Mitotic activity then ceases and the total population remains more or less constant up to 2 days after birth. Between 3 and 4 days, some degenerating cells are either eliminated from the seminiferous tubules, or become histologically unrecognizable, with the result that the population falls to about 100,000. This decline is followed at 4·5–5·5 days by a new and marked rise in the total population of germ cells brought about by a re-initiation of mitotic activity associated with the formation of the first generation of spermatogonia type-A.

These findings show a considerable measure of agreement with the results of previous studies involving counts of primordial germ cells per cross-section of seminiferous cords (Clermont & Perey, 1957; Harding, 1961; Hughes, 1962). The results of these quantitative studies finally invalidate the claims made by earlier authors (e.g. Hoven, 1914; Allen, 1918, 1949; Firket, 1920; Hargitt, 1926; see also Clermont & Perey, 1957) who stated that *all* primordial germ cells degenerate, definitive spermatogonia arising subsequently from supporting or other vegetative cells.

The observations made in the present study are equally in agreement with those of Roosen-Runge & Anderson (1959), who also used Chalkley's (1943) method to estimate the relative and absolute volumes occupied by interstitial and other tissue elements in the developing testis of the rat. They reported that the absolute volume of primordial germ cells increases from 15·5 days *p.c.* (designated as 15 days), reaches a peak at 19·5 days *p.c.* (cf. 20 days in the present study), and thereafter decreases gradually to about 4 days after birth. The latter decline, as determined in the present study, is somewhat less clear-cut and may possibly reflect a slight difference, between the two strains of rats, in the age at which degenerating gonocytes disappear from the testis.

The results of both studies indicate a steady increase in the volume of testicular tissue occupied by 'supporting' or 'basal' cells as development proceeds. The two estimates of the proportion of interstitial to other cells show a remarkably close agreement, even though different histological procedures were employed. Roosen-Runge & Anderson's material was stained by the PAS reaction, whereas, in the present study, sections were stained with haematoxylin and 'chromotrop 2R', the Leydig cells being differentiated by morphological rather than histochemical criteria.

Since similar methods have been used for the assessment of the populations of germ cells in both the ovary and the testis of the same strain of rat, a precise comparison between the two sexes is now possible. The primordial germ cells in both divide mitotically between 14·5 and 18·5 days *p.c.* In the female (Beaumont & Mandl, 1962), the maximum number of germ cells (*ca.* 75,000) is reached at 18·5 days *p.c.* (cf. some 140,000 at 20 days in the male). A difference between the sexes in the number of primordial germ cells is already apparent at 14·5 days *p.c.*, when the gonads first become histologically distinguishable. At this time, some 11,500 germ cells are present in the ovary, and *ca.* 20,000 in the testis. It is not known whether this discrepancy is due to a difference in the number of primordial germ cells originally segregated, or to a higher rate of mitotic activity in the genetically male embryo. Although Chiquoine (1954) and Mintz (1959) counted the numbers of migrating primordial germ cells in mouse embryos, neither of them attempted to distinguish between 'male' and 'female' specimens. Chiquoine, however, reported that although the population of germ cells increases rapidly between 8 and 12 days *p.c.*, the numbers were highly variable between embryos of the same age and even between littermates. Such variability may well be correlated with genetic sex.

The alternative suggestion, that the rate of proliferation of primordial germ cells varies between the two sexes, is not supported by a comparison of the calculated mitotic index *after* sex differentiation. At 14·5 days *p.c.*, for example, the estimated proportion of dividing germ cells is 5·3 per cent. in the female (Beaumont & Mandl, 1962) and 5·4 per cent. in the male; the following day, the indices are 6 and 4·7 per cent. respectively. These observations do not, however, eliminate the possibility that a high rate of mitotic activity sets in *earlier* in the male than the female, i.e. during the period of migration to the genital ridges and before the onset of sex differentiation.

From the time that mitotic activity ceases, the fate of the germ cells differs considerably between the two sexes. In the female, they pass into the prophase of meiosis and thus become definitive germ cells or oöcytes; in the male, on the other hand, they enter upon a relatively prolonged resting phase, during which they enlarge considerably. Degenerating germ cells subsequently appear in both the ovary and the testis. In the female, only about a third of the peak population of oöcytes persists to 2 days after birth. This depletion constitutes an irreversible loss of definitive germ cells. In the male, on the other hand, the numbers of primordial germ cells present at 4 days after birth represent some three-quarters of the maximum observed at 20 days *p.c.*

The latter finding contrasts with the inference drawn from many previous studies, that widespread degeneration of gonocytes occurs in the testis of the rat around the time of birth (see Clermont & Perey, 1957). Qualitative examination of the testes of animals aged 20 days *p.c.* or more, used in the present study, also suggested at first that large numbers of germ cells undergo lysis. However, the results of the quantitative studies show that there is no reduction in the total population of germ cells between 20 days *p.c.* and 2 days *p.p.* Furthermore, *all* gonocytes in specimens of comparable ages, studied by means of an electron microscope, appeared to be normal (Franchi & Mandl, unpublished observations); the first indications of the onset of lysis in a few of these cells become manifest at 2·5–3·5 days *p.p.*

These observations suggest that gonocytes may be particularly sensitive to osmotic or other changes during the last 2–3 days of foetal life and first few days after birth. They seem able to 'withstand' fixation in buffered osmic acid, but apparently incur considerable damage in fixatives (such as Bouin's aqueous fluid) used for routine histological purposes. It is perhaps significant that maximum radio-sensitivity has also been found to occur at this time (see below).

A detailed study of testicular development during the first week of post-natal life has indicated that the changes which precede the appearance of spermatogonia type-A are more complex than has hitherto been reported (e.g. Clermont & Perey, 1957; Harding, 1961; Hughes, 1962). Qualitative examination of the testes of animals aged 4·5–6·5 days *p.p.* suggested that the differentiation of definitive germ cells is a gradual process. It appears that the primordial germ cells first become 'transitional' morphologically, and migrate from the centre of the tubule towards the periphery. Substantial evidence for this migration has now been furnished by electron microscopic studies (Franchi & Mandl, unpublished), showing that cells in the centre of the seminiferous cords possess 'pseudopodia' extending towards, and frequently making contact with, the basement membrane.

The present observations indicate that the differentiation of 'transitional' cells occurs before the renewal of mitotic activity. For it is apparent that an inverse relationship exists between the numbers of primordial and 'transitional' germ cells, and that the rise in the number of the latter precedes the peak, occurring at 6 days *p.p.*, in both the absolute and relative numbers of mitoses. It is also evident that the initial rise in the population of 'transitional' cells precedes that of spermatogonia type-A. These conclusions are fully supported by Huckins's (1963) preliminary report on the developing testis of the rat. She describes the transformation of gonocytes into a 'darker cell type', and states that 'at the 5th post-natal day, these dark gonocytes begin to migrate towards the basement membrane and resume mitosis as they come to resemble type-A spermatogonia more and more'. Huckins also describes the lysis of 'a small percentage' of gonocytes which do not undergo this transformation. In the present study, the numbers of gonocytes diagnosed as 'lysing', in animals aged 4–6·5 days, averaged 12 per cent. of the total population. Although the time taken for degenerating gonocytes to 'disappear' from the tubules is unknown, the results of the quantitative analysis indicate that the number of cells actually eliminated represents a fairly small proportion of the whole population.

It has already been suggested that factors underlying the massive degeneration of germ cells in the foetal ovary may be twofold, namely genetic and environmental, whose relative importance it is difficult to assess (Beaumont & Mandl, 1962). In the case of the foetal testis, the situation is equally complex. As other authors have pointed out (e.g. Nebel, Amarose & Hackett, 1961; Huckins, 1963), alignment on the basement membrane appears to be a necessary prerequisite for further differentiation and/or division of germ cells. It would seem to be probable, therefore, that contact with the basement membrane, which brings the cell nearer to a capillary blood supply, also promotes oxygenation or access to some metabolite(s) which may be necessary for synthetic processes preceding cell division. In the rat and mouse, it seems that cells which fail to migrate to the periphery undergo degeneration and/or are eliminated, appearing subsequently in the epididymis (see Nebel *et al.*, 1961).

A comparison of the observations of Clermont & Perey (1957) on the rat, and those of Courot (1962) on the lamb, provides some grounds for the view that crowding of germ cells may induce degeneration. Courot reports that the histological appearance of the seminiferous cords remains practically unchanged between the completion of sex differentiation in the foetus, and the onset of the spermatogenetic cycle after birth. Both the primordial germ cells and the 'supporting' cells divide actively throughout the 'immature' phase and increase in number; at the same time, the relative volume of the testis occupied by seminiferous cords increases by 10 per cent. Thus the number of germ cells per 'tubular cross-section' (sections at 10μ; mean tubule diameter *ca.* 50μ) is merely maintained at a constant level, with values ranging between 0·4 and 1·1. It may be significant that in the rat, the average number of primordial germ cells per 'tubular cross section' (cut at 5μ; mean diameter of tubule *ca.* 65μ) is much higher between 15 and 21 days p.c. (mean 7·9; Clermont & Perey, 1957) than that recorded for the lamb at any stage of development. The subsequent reduction in the average numbers (2·2 at 1 day, and 1·2 at 4 days *p.p.*) is probably related to both the onset of degeneration and to the elongation of the seminiferous cords (see also Clermont & Huckins, 1961) leading to dispersal of germ cells.

It is also of interest to note that in the lamb, histochemical observations suggest that interstitial cells secrete little or no androgenic hormone (Ballie, 1960). In contrast, there is good evidence that the developing testis of the rat secretes a sex hormone (e.g. Price & Pannabecker, 1956; Deanesly, 1961). Roosen-Runge & Anderson (1959) further suggest that the various phases in the development of germ cells can be correlated with distinct stages in the differentiation of Leydig cells and hence with their functional activity.

Although these observations suggest that 'environmental' factors may play a major rôle in the degeneration of primordial germ cells, it is possible that genetic factors are also involved. The possibility that genetic deficiencies are expressed during the mitotic divisions which precede the formation of the first generation of spermatogonia type-A derives from several reports (e.g. Hargitt, 1926; Allen, 1949) that abnormal mitotic figures appear in the seminiferous cords about 1 week after birth. In the present study, however, so few abnormal germinal mitoses were observed in post-natal animals that no attempt was made to enumerate them.

The results of several studies (e.g. Beaumont, 1960; Starkie, 1961; Hughes, 1962) have suggested that the radiosensitivity of germinal elements in the foetal and neonatal testis reaches a peak during the resting or growth phase of the primordial germ cells. The observation that radiosensitivity decreases at times when mitotic activity is high, i.e. before and after the resting phase, is contrary to what would be expected in the light of general radiobiological principles. Moreover, the results of the present investigation have demonstrated that this period of high sensitivity is *not* associated with a high rate of spontaneous degeneration, as had previously been supposed. A series of experiments is now planned to study some aspects of the metabolic activity of the resting germ cells, and to investigate early post-irradiation changes. This approach may contribute to the understanding of the factors which influence both spontaneous and irradiation-induced degenerative changes.

SUMMARY

1. A cytological and quantitative study was made of the testes of foetal and neonatal rats. The population of germ cells was estimated for a total of 111 specimens. A volumetric method (Beaumont & Mandl, 1962) was used for animals aged 14·5 days *p.c.* to 4·5 days *p.p.* Counts were made of the numbers of dividing germ cells in foetal testes. Numbers of normal, degenerating and dividing gonocytes, 'transitional' cells and spermatogonia type-A were estimated from differential counts made on specimens aged 3–6·5 days *p.p.*

2. The primordial germ cells divide mitotically up to the age of about 18·5 days *p.c.* Thereafter they remain at interphase for a period of 6–8 days, during which they enlarge considerably. Although many gonocytes appear to undergo degeneration during this resting period, few 'disappear' from the testis until 2–4 days after birth. Differentiation of gonocytes into definitive germ cells sets in about 4 days *p.p.* The formation of spermatogonia type-A is preceded by the appearance of 'transitional' cells and a renewal of mitotic activity.

3. The total population of primordial germ cells rises from about 20,000 at 14·5 days *p.c.* to reach a plateau of 120,000–140,000 between 20 days *p.c.* and 2 days *p.p.* The number of gonocytes then declines to *ca.* 50,000 at 6–6·5 days after birth. This decrease is due to (*a*) the elimination of degenerating germ cells, and (*b*) the transformation of gonocytes into definitive germ cells. With the re-initiation of mitotic activity, which accompanies germinal differentiation, the total population of germ cells increases to *ca.* 200,000 at 6–6·5 days, of which spermatogonia type-A number between 50,000 and 90,000.

RÉSUMÉ

Étude quantitative des gonocytes primordiaux chez le rat male

1. On a fait une étude cytologique et quantitative des testicules de foetus et de nouveau-nés de rat, et on a évalué la population de gonocytes pour un total de 111 individus. Une méthode volumétrique (Beaumont et Mandl, 1962) a été utilisée pour les animaux âgés de 14 jours 1/2 *post coitum* à 4 jours 1/2 *post partum*. On a dénombré les gonocytes en division dans les testicules foetaux. Le nombre de gonocytes normaux, en dégénéréscence et en division, de cellules 'de transition' et de spermatogonies de type A a été évalué à partir de comptages différentiels faits sur des individus âgés de 3 à 6 jours 1/2 *p.p.*

2. Les gonocytes primordiaux se divisent par mitose jusqu' à l'âge d'environ 18 jours 1/2 *p.c.* Ensuite, ils demeurent en interphase pendant une période de 6 à 8 jours, au cours de laquelle ils grossissent considérablement.

Bien que beaucoup de gonocytes paraissent subir une dégénérescence pendant cette période de repos, peu d'entre eux 'disparaissent' du testicule jusqu'à 2–4 jours après la naissance. La différenciation des gonocytes en cellules germinales définitives commence environ 4 jours *p.p.* La formation des spermatogonies de type A est précédée par l'apparition de cellules 'de transition' et un renouveau de l'activité mitotique.

3. La population totale de gonocytes primordiaux s'élève à partir de 20.000 environ à 14 jours 1/2 *p.c.* pour atteindre un plateau de 120 à 140.000 entre 20 jours *p.c.* et 2 jours *p.p.* Puis le nombre de gonocytes diminue ensuite aux environs de 50.000, 6 à 6 jours 1/2 après la naissance. Cette décroissance est due à (*a*) l'élimination de gonocytes en dégénérescence, et (*b*) la transformation de gonocytes en cellules germinales définitives. Avec la reprise d'activité mitotique qui accompagne la différenciation germinale, la population totale de gonocytes atteint environ 200.000 à 6 jours–6 jours 1/2, le nombre de spermatogonies de type A y étant compris entre 50.000 et 90.000.

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