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# Genetic control of the kinetics of mouse spermatogenesis

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SUMMARY

The kinetics of spermatogenesis in the mouse, and the possible genetic controls, have been investigated in different genotypes by means of the method of velocity sedimentation at unit gravity to separate testis cells and nuclei labelled with tritiated thymidine. The progression of the radioactivity through different sedimentation classes of cells provides a measure of their kinetics. The results demonstrate kinetic differences at the stage of spermatocyte differentiation between the C57BL/6 and AKR inbred strains of *Mus musculus musculus* and in randombred mice of the subspecies *Mus m. molossinus*. These kinetic differences are controlled by genetic factors which are not linked to the *Y* chromosome. The relevant autosomal alleles of C57BL/6 are dominant over those of AKR in $F_1$ hybrids.

## 1. INTRODUCTION

The differentiation of male germinal cells in the mouse and other mammals investigated in detail is highly synchronous (Clermont, 1972). That is, the time intervals between various stages in the maturation process are well defined and may be precisely and reproducibly measured (Oakberg, 1956*b*; Clermont & Trott, 1969). The stages have been conventionally defined by the microscopic appearance of the cells (Oakberg, 1956*a*). However, physical parameters such as the sedimentation velocity of the cells (Lam, Furrer & Bruce, 1970) and nuclei (Meistrich & Eng, 1972) are also quite useful for characterizing stages of differentiation. Measurements of kinetics of differentiation utilize the fact that the last DNA synthetic period, in the preleptotene primary spermatocyte (Monesi, 1962), accounts for about one-half of the testicular incorporation of radioactive thymidine. Since there is negligible turnover of the DNA, the kinetics of differentiation may be followed by radioautography of tissue sections or by the sedimentation rates of the labelled cells.

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We report here that the latter method can be readily used to measure differences in the kinetics of spermatogenesis between different genotypes of mice and the inheritance pattern of the component phenotypes can thus be studied.

## 2. MATERIALS AND METHODS

### (i) *Mice*

Males of the laboratory mouse *Mus musculus musculus* were used from the following inbred strains (sublines of the Institute for Cancer Research, Philadelphia): A/HeNIcr, AKR/NIcr, BALB/cAnNIcr, CBA/HIcr, C3HfB/HeNIcr, C57BL/6JNIcr, and DBA/2NIcr (all will be referred to by strain abbreviations). A number

Table 1. *Chromosomal constitution of male mice used in spermatogenesis studies*

| | Chromosome | | | |
|---|---|---|---|---|
| | | | Autosomes | |
| | *X* | *Y* | | |
| C57* | C57 | C57 | C57 | C57 |
| AKR* | AKR | AKR | AKR | AKR |
| C57-$Y^{AKR}$ | C57 | AKR | C57 | C57 |
| $F_1$ (C57 ♀ × AKR ♂) | C57 | AKR | C57 | AKR |
| $F_1$ (AKR ♀ × C57 ♂) | AKR | C57 | C57 | AKR |
| '$F_1$' (AKR ♀ × C57-$Y^{AKR}$ ♂) | AKR | AKR | C57 | AKR |
| $F_2$ | (C57 or AKR) | AKR | (C57 or AKR) | (C57 or AKR) |

* The sublines of these strains were C57BL/6JNIcr and AKR/NIcr, respectively.

of congenic derivatives of the C57 strain were created in order to test for possible spermatogenesis-controlling factors on the *Y* chromosome: a male of another strain was mated to a C57 female and the male progeny were repeatedly backcrossed to C57 females for more than seven generations, so as to obtain a series of strains presumably differing from C57 only by their *Y* chromosomes. Thus, for example, males of the strain designated C57-$Y^{AKR}$, used in the present study, have over a 99 % probability of each of their autosomal and *X* chromosome genes being the same as those of the C57 strain (barring the unlikely possibility of genetic recombination between parts of the *X* and *Y*), while the *Y* chromosome is of the AKR type. Conventional $F_1$ hybrids were obtained by reciprocal crosses of (C57 ♀ × AKR ♂) and (AKR ♀ × C57 ♂). Another kind of '$F_1$' hybrid, from the cross between (AKR ♀ × C57-$Y^{AKR}$ ♂), was also created as one means of screening for possible genetic controls on the *X* chromosome: males from this cross differ from those obtained from the $F_1$ (C57 ♀ × AKR ♂) cross by their *X* chromosomes only. The '$F_1$' males also differ from those of the $F_1$ (AKR ♀ × C57 ♂) cross by their *Y* chromosomes. The $F_2$ generation was produced by matings between $F_1$ (C57 ♀ × AKR ♂) individuals. The chromosome composition of all these animals is outlined in Table 1.

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*Mus. m. molossinus*, a subspecies interfertile with *Mus m. musculus*, was obtained from Dr Michael Potter of the National Cancer Institute, Bethesda, Md. These mice were maintained as a randombred closed colony. The mice designated C57-$Y^{molossinus}$ were produced analogously to C57-$Y^{AKR}$ except that only three generations of backcrossing were performed.

The animals were kept in a light-controlled room (14 h light, 10 h dark) with 3–5 males per cage. Mice between 9 and 15 weeks of age were used in all experiments. The animals were sacrificed by cervical disolocation.

### (ii) *Cell separation and counting*

Separation of testis cells and nuclei was performed using the 'Staput' method of velocity sedimentation at unit gravity. The principles involved have been described by Miller & Phillips (1969) for separations of other cell types. A gradient of bovine serum albumin in phosphate buffered saline (PBS) (0·026 % albumin per vertical mm) was utilized as described previously for spermatogenic cell separations (Lam *et al.* 1970). Except as noted, cell suspensions were prepared by mechanical means, for convenience. In one series of experiments, trypsin and DNase were employed to prepare the suspensions (Meistrich, 1972). Cells were counted with a Coulter counter (Model F) with a 70 $\mu$m aperture.

### (iii) *Preparation and separation of testis nuclei*

Nuclei were prepared from a cell suspension with the detergent cetrimide (hexadecyltrimethylammonium bromide, Eastman Kodak). Testis tubules were first teased apart, using fine forceps, in 5 ml PBS/testis and then cooled to 4 °C. Cells were pelleted at 320 g (15 min) and resuspended in 5 mg/ml cetrimide in 1 mM-$CaCl_2$ at pH 5·2. The suspension was vortexed at high speed for 20 sec, then passed through an 80 $\mu$m screen to remove any undissociated tubule material. This procedure yielded an average of $3 \cdot 5 \times 10^7$ nuclei per testis of mice of the C57BL/6JNIcr strain. The nuclei appear to be free of cytoplasm and have only the inner nuclear membrane remaining, as established by electron microscopy (Loir & Wyrobek, 1972). Under phase-contrast, the nuclei appear fully rounded and have the same size, shape, and grossly detectable heterochromatin pattern as nuclei of intact cells.

In each experiment, between $3 \times 10^7$ and $7 \times 10^7$ nuclei were separated by velocity sedimentation at unit gravity for 5 h at 4 °C in a Ficoll gradient. A 1–2·2 % linear gradient of Ficoll (gradient of 0·027 % Ficoll per vertical mm) was prepared in 1 mM $CaCl_2$ and 4 mg/ml cetrimide pH 5·2. Other procedures for separation were as described by Lam *et al.* (1970).

### (iv) *Radioactive labelling and counting*

Testicular cells were labelled by injecting mice intraperitoneally with 1 $\mu$Ci/g body weight of [$^3$H]thymidine (TdR, specific activity *c.* 20 Ci/mM, Amersham-Searle). Labelled cells and nuclei were filtered and washed as described previously (Lam *et al.* 1970), except that Whatman GF/C filters were used.

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### (v) *Histological methods*

Testes were fixed in Zenker-formol (4 % formaldehyde) for 4 h. Sections were cut and stained with the periodic acid–Schiff reaction and counterstained in hematoxylin. Cells and stages of the cycle of the seminiferous epithelium were identified according to the criteria of Oakberg (1956*a*).

## 3. RESULTS

### (i) *Preliminary observations*

In order to ascertain if the relative numbers of cells at different stages of development were the same in the various inbred strains, cell suspensions were prepared mechanically from the A, AKR, BALB/c, CBA, C3Hf, C57, and DBA strains. Staput separations were performed and the distribution of cells was

Table 2. *Assignment of peaks of radioactivity to spermatogenic cells and nuclei*

| Mean sedimentation velocity (mm/h) | Cell type |
|---|---|
| 9·8* | Pachytene spermatocyte ($\beta$)† |
| 6·5* | Secondary spermatocyte and binucleate spermatid ($\gamma$) |
| 4·4* | Early spermatid ($\delta$) |
| 2·4* | Damaged pachytene spermatocyte ($\theta$) |
| 1·1* | Spermatid nucleus ($\kappa$) |
| 2·40‡ | Pachytene spermatocyte nucleus ($\pi$) |
| 1·46‡ | Secondary spermatocyte nucleus |
| 1·01‡ | Early spermatid nucleus ($\kappa$) |

* In BSA gradient.
† Greek letters represent notations used in Lam *et al.* (1970).
‡ In Ficoll gradient.

determined by counting cells with an electronic cell counter. The distribution of cell number as a function of sedimentation velocity was the same for all strains tested and indistinguishable from that of the $BC3F_1$ (C57 ♀ × C3H ♂) hybrids (Lam *et al.* 1970) and C57BL/6J (Meistrich, 1972). Furthermore, the sedimentation velocities of spermatocytes and spermatids from the various strains were identical.

Next, kinetic studies of spermatogenesis were carried out by analysis of the sedimentation velocity profile of $^3$H-TdR-labelled cells. Mechanically prepared cell suspensions from the various strains were analysed at 8 and 12 days after labelling. No striking differences were seen in the 8-day patterns, although the peak of labelled pachytene spermatocytes in the AKR strain did show a slightly lower sedimentation rate than that of any of the other strains, indicating that the rate of spermatocyte differentiation is lower in that strain. This difference was more apparent at 12 days, when there was a striking difference between the profiles of labelled cells obtained from the C57 and AKR mice (Text-fig. 1). The identification

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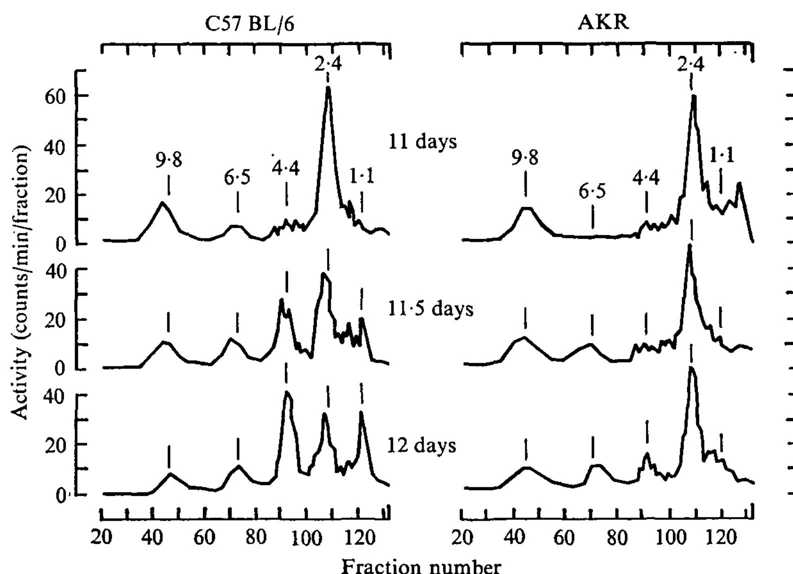
of the various peaks obtained was based on criteria previously used (Lam *et al.* 1970; Meistrich & Eng, 1972; Meistrich, 1972; Meistrich, Bruce & Clermont, 1973*a*) and is presented in Table 2. These curves were analysed quantitatively by calculating the percentage of total radioactivity in the various peak regions. The fraction of label in the peak at 4·4 mm/h (early spermatids) provides a measure of the progress of spermatogenesis and is tabulated in Table 3. The profiles from the other inbred strains of *Mus m. musculus* were similar to that of C57, as indicated by the data




Text-fig. 1. Velocity sedimentation profile of radioactivity in mechanically prepared testis cell suspensions 11, 11·5 and 12 days following the injection of [$^3$H]thymidine. Migration of counts from the 9·8 to the 6·5 and 4·4 mm/h peaks is delayed in the AKR mouse relative to the C57BL/6. (In this and subsequent figures, both upper and lower abscissae are calibrated in fraction numbers.) The sedimentation velocities of the peaks of radioactivity are indicated.

in Table 3; these strains were not investigated further. *Mus m. molossinus*, on the other hand, presented a radioactivity profile similar to that of AKR. More detailed quantitative data have been obtained which indicate that the interval between the S-phase of the primary spermatocyte and the meiotic divisions is 0·7 day longer in *Mus. m. molossinus* than in C57.

### (ii) *Comparison of kinetics of spermatogenesis in C57 and AKR*

The changes in sedimentation velocity of labelled spermatogenic cells are most pronounced and reproducible as the cells go through the meiotic divisions. Therefore, the sedimentation profiles of cells labelled *in vivo* by injection of $^3$H-TdR were examined during the period of 11–12 days after administration of $^3$H-TdR (Text-fig. 1). The greater heights of the 9·8 and 2·4 mm/h peaks in AKR and of the 4·4 and 1·1 mm/hr peaks in C57 indicate that the labelled cells in C57 go

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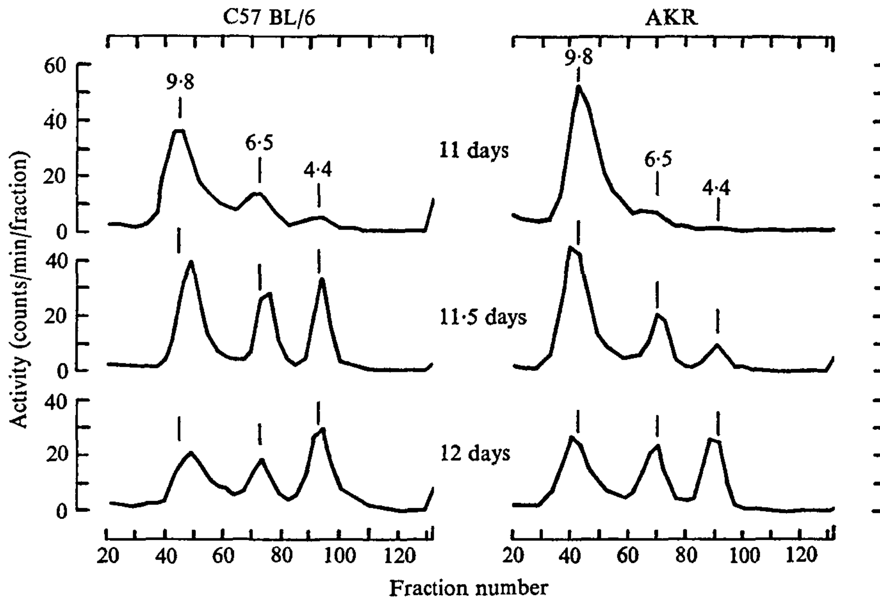
through meiosis faster than those in AKR. There is a delay of slightly less than one-half day in the development of spermatocytes in AKR compared to C57.

As these cell preparations contain a mixture of intact and damaged cells (Meistrich, 1972), we wished to confirm the results using both suspensions of intact cells as well as suspensions of isolated nuclei. The intact cells were obtained by trypsinization and the isolated nuclei with the detergent cetrimide. Velocity sedimentation profiles of radioactivity using both these preparations were obtained




Text-fig. 2. Velocity sedimentation profile of radioactivity in trypsinized testis cell suspensions 11, 11·5 and 12 days following injection with [$^3$H]thymidine. Migration of counts from the 9·8 to the 6·5 and 4·4 mm/h peaks is delayed in the AKR mouse relative to the C57BL/6.

for suspensions prepared at 11, 11·5 and 12 days after labelling. The profiles shown in Text-figs. 2 and 3 demonstrate the same lag for the AKR strain. From the fraction of label in the 4·4 mm/h peak we can calculate the lag in the AKR to be 0·3 days (cf. Meistrich, Eng & Loir, 1973*b*, fig. 4).

The possibility that lower steroid levels in AKR (Metcalf, 1960) might be responsible for this difference in kinetics was tested by injecting Prednisolone (meticortelone, obtained as Prednisolone Sodium Hemisuccinate, Schering Ltd.) at doses of 0·01, 0·1 and 1 mg per day intramuscularly for 16 days, beginning 4 days prior to administration of $^3$H-TdR. This treatment had no effect on the patterns of labelled cells at 12 days after injection of $^3$H-TdR.

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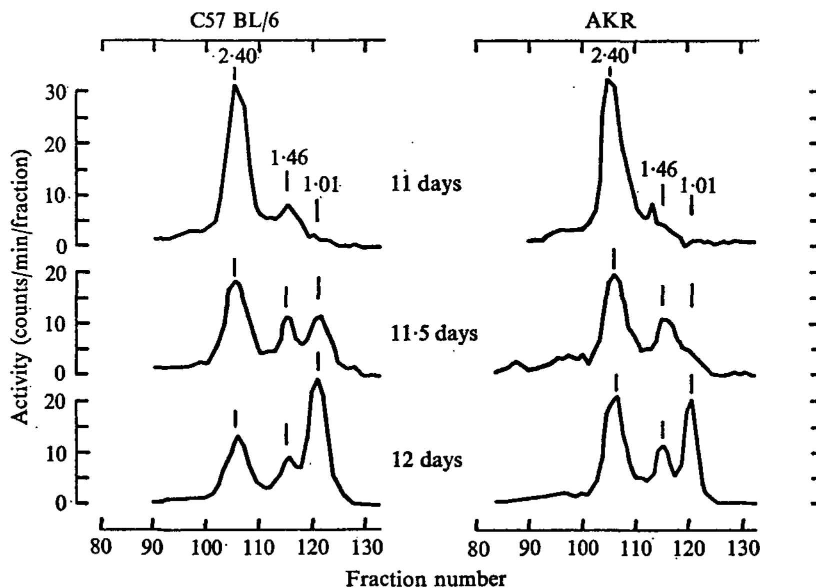
### (iii) *Genetic studies*

The effects of the genotype on the kinetics of spermatogenesis was further examined (Table 3) by injecting mice of the indicated genotypes with $^3$H-TdR and sacrificing them 12 days later. Cell suspensions were prepared mechanically and separated by velocity sedimentation. From the radioactivity profiles, the fraction of radioactivity in the 4·4 mm/h peak was calculated (Table 3). The cumulative distribution functions of this parameter, presented graphically in Text-fig. 4, are shown for individual animals.




Text-fig. 3. Velocity sedimentation profile of radioactivity in detergent prepared testis nuclei 11, 11·5 and 12 days following the injection of [$^3$H]thymidine. Migration of counts from the 2·4 to the 1·46 and 1·01 mm/h peaks is delayed in AKR relative to C57BL/6.

The effect on the kinetics of genes linked to the *Y* chromosome was shown to be negligible, by comparison of the data from C57 with C57-Y$^{AKR}$, and from $F_1$ (AKR ♀ × C57 ♂) with '$F_1$' (AKR ♀ × C57-Y$^{AKR}$ ♂). Genes linked to the *X* chromosome may contribute to the different kinetics in C57 and AKR; this was shown by comparisons of $F_1$ (C57 ♀ × AKR ♂) with '$F_1$' (AKR ♀ × C57-Y$^{AKR}$ ♂) or, since the source of the *Y* is irrelevant, with $F_1$ (AKR ♀ × C57 ♂) as well. The data in Table 3 show that in $F_1$ hybrids with the *X* chromosome from C57 *vs.* AKR the fractions of label in the 4·4 mm/h peak are $26·8 \pm 1·8$ and $23·4 \pm 0·8$. Thus although the latter value is smaller, the difference is only barely significant ($P = 0·04$). Furthermore, the $F_1$ with the *X* from the C57 parent is indistinguishable from the C57 parent, indicating that in general the autosomal genes from AKR are recessive to C57. In the $F_2$ generation, the value of $23·4 \pm 1·3$ is lower (but not significantly, $P = 0·14$) than that of the C57 parent and is consistent with 22·8 which is 75%

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of the C57 value and 24 % of the AKR value, as expected for recessive alleles in AKR. The estimated population standard deviation of the values of the $F_2$ is significantly higher than that of the parental mice and some of those of the $F_1$ generation. Using a formula for estimating the minimum number of genes involved (Falconer, 1963), this high standard deviation in $F_2$ results in a minimum or low estimate of only one or two genes involved. The fact that several of the $F_2$ mice are similar to the AKR parent (Text-fig. 4) makes it unlikely that many independently segregating genes are involved.

Table 3. *Kinetics of spermatogenesis in various genotypes of mice as determined by the fraction of total radioactivity in the δ peak (fractions 86–101) at 12 days after injection of* $^3$*H-TdR*

| | | Fraction of label in 4·4 mm/h peak | |
|---|---|---|---|
| Genotype or cross | No. of mice | Mean ± s.e. | Population standard deviation ± standard deviation of s.d.* |
| A | 1 | 26·2 | — |
| Balb/c | 1 | 22·8 | — |
| CBA | 1 | 22·4 | — |
| C3Hf | 1 | 23·7 | — |
| DBA | 1 | 21·5 | — |
| AKR | 5 | 14·4 ± 1·1 | 2·5 ± 0·8 |
| C57 | 5 | 25·6 ± 1·4 | 3·1 ± 1·0 |
| C57-$Y^{AKR}$ | 5 | 25·5 ± 1·0 | 2·2 ± 0·7 |
| $F_1$ (C57 ♀ × AKR ♂) | 8 | 26·8 ± 1·8 | 5·0 ± 1·2 |
| $F_1$ (AKR ♀ × C57 ♂) | 8 | 23·3 ± 0·8 | 2·2 ± 0·6 |
| '$F_1$' (AKR ♀ × C57-$Y^{AKR}$ ♂) | 3 | 23·9 ± 2·5 | 4·3 ± 1·8 |
| $F_2$ | 20 | 23·4 ± 1·3 | 5·9 ± 0·9 |
| C57, C57-$Y^{AKR}$ } Data pooled | 10 | 25·6 ± 0·8 | 2·5 ± 0·6 |
| AKR ♀ × C57 ♂, AKR ♀ × C57-$Y^{AKR}$ ♂ } Data pooled | 11 | 23·4 ± 0·8 | 2·7 ± 0·6 |
| *Mus. m. molossinus* | 1 | 13·3 | |

* Estimated by the formula for a normal distribution: $\sigma_s = \sigma/\sqrt{(2n)}$, where $\sigma_s$ is the standard deviation in the sample standard deviation, $\sigma$ is the population standard deviation and $n$ is the number of measurements.

No extensive attempts were made to correlate the kinetics of spermatogenesis in the $F_2$ generation with other genetic markers. It was noted that those albino mice analysed showed a lower amount of label in the 4·4 mm/h peak than the black mice, but the difference was not significant.

Possible linkage of genes controlling the observed differences in kinetics of spermatogenesis with the $Y$ chromosome of *Mus m. molossinus* was tested and not confirmed in a sample of several C57-$Y^{\text{molossinus}}$ mice derived from two different *Mus m. molossinus* males; the results were indistinguishable from those of C57.

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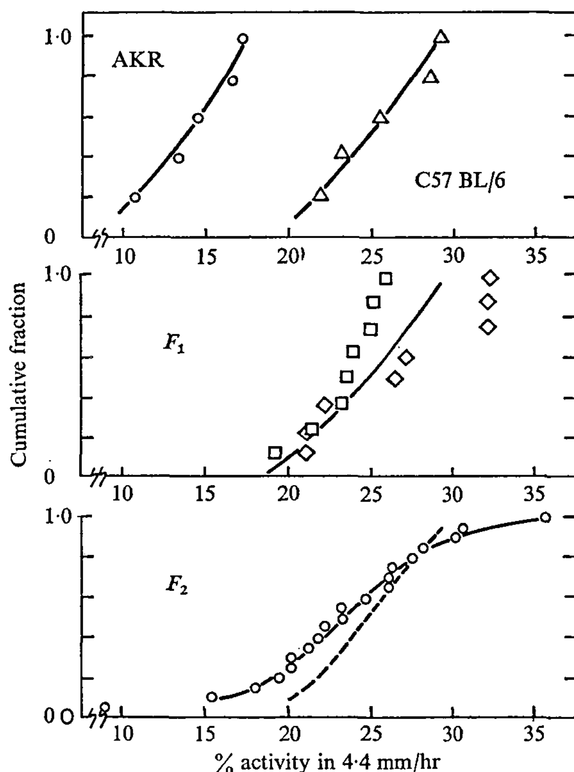
(iv) *Histological observations*

The spatially fixed cell relationships in the seminiferous epithelium can permit determination of the relative temporal relationships during the different parts of the developmental sequence (Leblond & Clermont, 1952; Oakberg, 1956*a*; Roosen-Runge, 1962). We have already demonstrated that there is a genetically controlled




Text-fig. 4. Cumulative distribution of mice with a given portion of radioactivity in the 4·4 mm/h peak at 12 days following injection of [$^3$H]thymidine. Upper panel: AKR and C57BL/6. Middle panel: $F_1$ (C57BL/6 × AKR), ◇, $F_1$ (AKR × C57BL/6), □, Lower panel: $F_2$ of AKR × C57BL/6. Dashed line represents data for C57BL/6 above. Each symbol represents results for one animal.

alteration in the time of development of S-phase spermatocytes to the meiotic divisions. If the development of other cells is proportionately retarded in AKR we would expect no changes in the cell associations present but merely an increase in the time required for a cycle of the seminiferous epithelium. On the other hand, if the intervals between different points in development vary independently between strains, then there must be alterations in the cell associations observed.

The stages between VII and X (Oakberg, 1956*a*) provide the opportunity for precise observation of cell development in three generations of germinal cells. Striking changes occur in (1) the chromatin pattern in the spermatocytes during the preleptotene to leptotene transition, (2) the shape, nuclear staining and

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presence of the centromeric heterochromatin body during the transition of spermatids from step VII to step X, and (3) the formation of the residual body in the cytoplasm of the spermatozoa (Firlit & Davis, 1965; Perey, Clermont & Leblond, 1961), and finally the disappearance of these cells from the seminiferous epithelium.

The development of the young spermatocytes and young spermatids shows the same spatial relationship in tubules of both the C57 and AKR strains. An average of 43 tubular cross-sections in stages VII–X were examined in each of three AKR and three C57 mice. The cell association in each tubule was graphically represented

Table 4. *Number of tubules at various stages of cycle with spermatozoa present or absent*

| Strain | Presence of spermatozoa | Stage of cycle* | |
|---|---|---|---|
| | | Stage 8 | Stage 9 |
| C57 | +† | 160‡ (75 ± 2 %)§ | 2 (2 ± 1 %) |
| | ±‖ | 16 (8 ± 2 %) | 9 (7 ± 2 %) |
| | −¶ | 36 (17 ± 3 %) | 121 (92 ± 2 %) |
| AKR | + | 175 (95 ± 3 %) | 52 (31 ± 3 %) |
| | ± | 6 (3 ± 3 %) | 46 (28 ± 5 %) |
| | − | 4 (2 ± 1 %) | 69 (41 ± 5 %) |

* Determined by morphology of early spermatids.

† Spermatozoa present, appearance typical of stage 7d or 8a defined by Perey, Clermont & Leblond (1961).

‡ Values represent total number of tubules in each category from sections of testes from six mice for each strain.

§ Percentage of tubules at stages 8 or 9 with indicated content of spermatozoa and standard error. The standard error represents the variation between mice.

‖ Only a few (1–5 per tubule cross-section) residual spermatozoa present.

¶ No spermatozoa present.

by a point, the abscissa value of which was the degree of spermatocyte development expressed in arbitrary numerical units and the ordinate value of which was the step of spermatid development. Since there are slight variations in cell associations (e.g. mid-preleptotene spermatocytes are occasionally seen in tubules with step 7 or step 9 spermatids in addition to the usual association with step 8 spermatids) (Clermont, 1972) it was necessary to average the data. A least-squares linear fit of the graphical data from each mouse demonstrated that the midpoint of the preleptotene to leptotene transition was associated on the average with spermatids in the latter half of step 9 of development. This association was the same in both strains examined within the standard error (between mice of a given strain) of 0·2 of a stage. Translated on to a temporal scale based on the duration of stage IX (Oakberg, 1956*a*, *b*) this result indicates that the stages of development of the spermatids associated with the preleptotene spermatocytes are the same to within 3 h in both strains of mice. The constancy of cell associations implies that spermatid development in AKR is slowed to the same degree as has been demonstrated for spermatocyte development.

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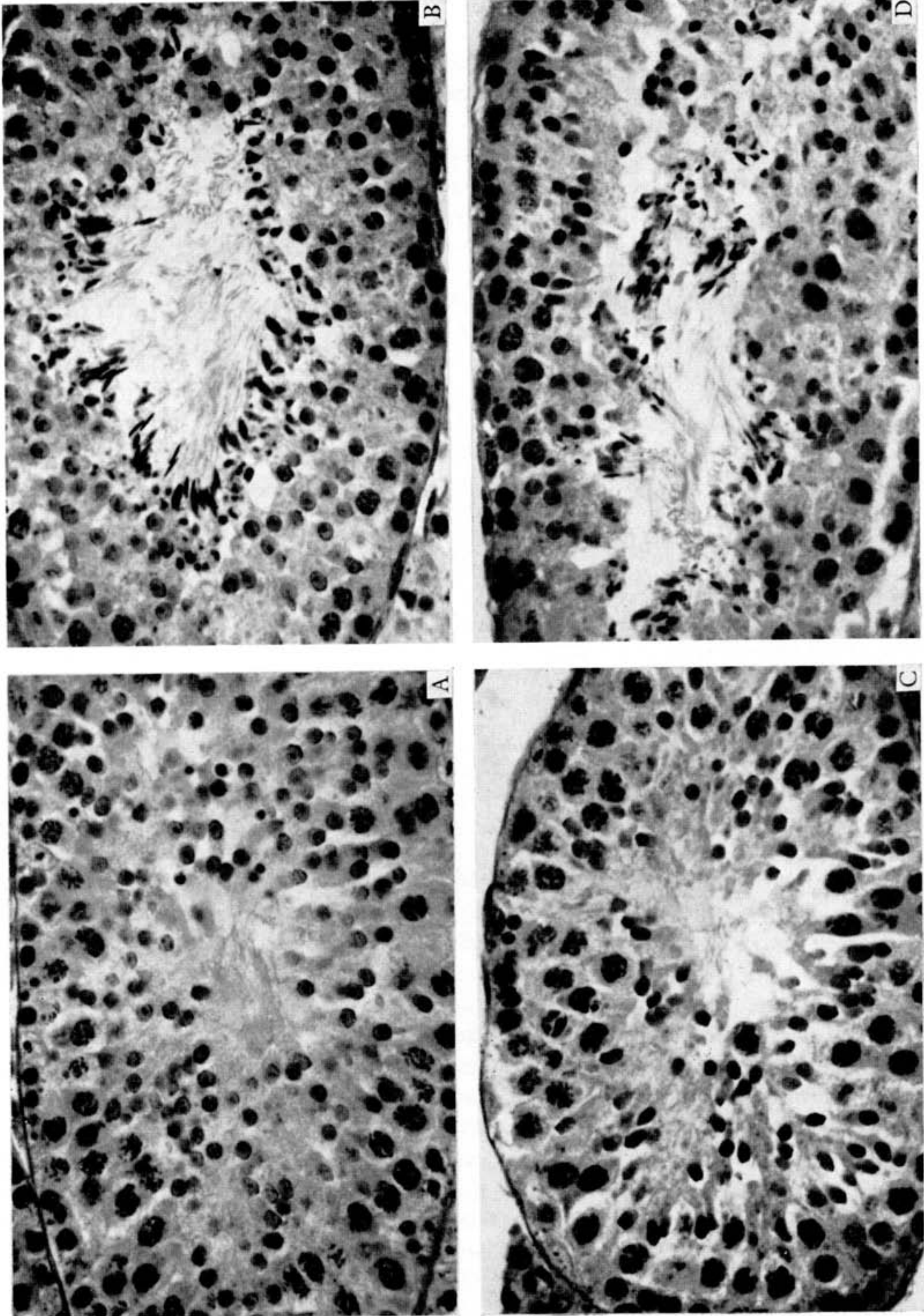


Cross-sections of mouse testis tubules (×420). (*a*) C57 stage VIII. No spermatozoa. (*b*) AKR stage VIII. Spermatozoa present. (*c*) C57 stage IX. No spermatozoa. (*d*) AKR stage IX. Spermatozoa present.

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On the other hand, the associations between the spermatozoa and spermatids are slightly different in mice of the AKR and C57 strains. Spermatozoa were more frequently seen in tubules at stages VIII and IX in AKR mice than in C57 (Table 4). Examples of the presence of spermatozoa at stage IX in AKR and their absence at stage VIII in C57 are shown in Plate 1. The delay in maturation and release of spermatozoa in AKR mice as opposed to C57 is estimated to be an additional 5 h over that resulting from the lengthening of the cycle of the seminiferous epithelium.

## 4. DISCUSSION

The kinetics of spermatogenic cell differentiation in mammals are precisely controlled. These kinetics have been conventionally measured by following the progression of thymidine labelled testis cells by autoradiography (Clermont, 1972). We have recently shown that equivalent results may be obtained under experimental conditions by velocity sedimentation analysis of these cells (Meistrich *et al.* 1973*a*; Meistrich *et al.* 1973*b*). In the present study, using three different preparation methods, we have demonstrated that equivalent kinetic data are obtained providing further support for the validity of the technique.

Although the kinetics of spermatogenesis do not vary between animals within an individual strain, there are differences between strains. Clermont (1972) found with autoradiographic techniques that there is a significant difference of about 8 % between the kinetics of spermatogenic cell differentiation of different strains of rats. We have reported here a similar phenomenon between strains of mice and have followed the genetics of this trait.

The present data demonstrate that spermatogenesis in AKR mice and mice of the subspecies *Mus m. molossinus* proceeds 3 % and 6 % slower, respectively, than in C57 mice and several other inbred strains of *Mus m. musculus*. Genes on the $Y$ chromosome have no significant effect on the spermatogenic parameters investigated here. By contrast, $Y$ linked factors of the mouse seem to be involved, in as yet undefined ways, in male sex determination (Welshons & Russell, 1959), and spermatozoan form (Beatty, 1969) and fertility (Krzanowska, 1969). There is a probable contribution to kinetic differences of genes on the $X$ chromosome. Autosomal genes are involved (from data in the $F_1$ generation) and those of C57 are generally dominant over those of AKR. From the data of the $F_2$ generation, we conclude that there is a small number of independently segregating genes controlling the process.

The mechanism for the genetic regulation of the kinetics of spermatogenesis is not known. The present experiments and all others which have tested the effects of gonadotrophic or steroid hormones have yielded negative results (Clermont & Harvey, 1965; Desclin & Ortavant, 1963; Go, Vernon and Fritz, 1971). The only physiological factor known to affect the kinetics of spermatogenesis is temperature (Meistrich *et al.* 1973*b*). Thus testicular temperature differences could conceivably be responsible for the variation in kinetics of spermatogenesis between strains, but this has not been examined in the strains studied here. Alternatively, there may



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be an intrinsic difference expressed within the differentiating germ cells of different genotypes; a test of this hypothesis would require an *in vitro* system for studying spermatocyte differentiation.

Histological observations have shown that there is a slight but measurable change in the cell associations observed in the seminiferous epithelium between strains which show different kinetics. However, the only difference observed was the stage of the cycle at which release of the mature spermatozoa (spermiation) occurred. Other experiments nevertheless indicate that spermiation might be affected by hormonal and damaging factors while the associations at earlier stages are insensitive to similar treatments (Clermont & Morgenthaler, 1955; Chowdhury & Steinberger, 1964; Oakberg, 1956*b*; Vitale-Calpe & Burgos, 1970). The lack of any difference in an earlier stage in our experiments suggests that these cell associations are indeed fixed, but this should be further tested if an example of more extreme strain differences in kinetics is uncovered.

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