Chromosoma (Berl.) 85, 427–437 (1982)

CHROMOSOMA

Meiosis in the Foetal Mouse Ovary

I. An Analysis at the Light Microscope Level Using Surface-Spreading

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Abstract. The identification and progression of the prophase stages of meiosis in the mouse foetal ovary are reported, from d 13 of gestation to d 1 postpartum. Air-dried Giemsa-stained oocyte preparations are compared with surface-spread silver-stained cells. The latter method allows a more detailed quantitative analysis of the pachytene stage. Numbers of synaptonemal complexes can be counted, and the degree of synapsis determined. The progression of cells appears to be relatively synchronous, in agreement with previous reports. The activity of nucleolar organisers, in particular one associated with the shortest synaptonemal complex (chromosome No. 19) is described. At late pachytene the lateral elements of the No. 19 bivalent desynapse precociously with apparent nucleolar involvement.

Introduction

A number of investigations have been made on oogenesis in the mouse foetal ovary using squash or sectioning techniques (Borum, 1961, 1966; Bakken and McClanahan, 1978). The timing of meiosis in the mouse oocyte by ^{3}H-autoradiography has also been carried out (Peters et al., 1962). More recently, air-drying methods have been applied to mouse oocytes (Jagiello and Fang, 1979) for chromosomal analysis.

In the present report, the sequence of oocyte maturation in the mouse foetal ovary is described as seen in surface-spread silver-stained preparations. Surface-spreading has been used previously for spermatocyte chromosome analysis at the light microscope level (Dresser and Moses, 1979; Fletcher, 1979) and with minor modification, the technique has now been found to give excellent results on oocytes. The method is simple, quick and could be applied to oocytes of any mammalian species. Moreover, it provides a degree of chromosomal detail in the prophase of meiosis which cannot be obtained with the air-drying technique.

0009-5915/82/0085/0427/$02.20

In the second paper of this series (Speed and Chandley, in preparation) data will be presented on the various kinds of synaptic irregularities seen in oocytes as they progress through the pachytene stage and compare the findings for two strains of mouse, one (CBA) showing a strong maternal age effect for aneuploidy (Gosden, 1973; Fabricant and Schneider, 1978) the other (Swiss) showing negligible spontaneous aneuploidy levels (pers. comm. J.D. Brook). The relevance of these observations to the "production line" hypothesis of oocyte development (Henderson and Edwards, 1968) will be discussed.

Materials and Methods

Mice of the Swiss albino random-bred strain were used in this initial investigation. To obtain foetuses of increasing gestational age, matings were set up, two females being placed with one male per cage. The morning on which a vaginal plug was seen was recorded as Day 0 of gestation.

The technique to prepare foetal oocytes by surface spreading is a modification of the method of Fletcher (1979). From each female foetus, both ovaries were removed and placed in Dulbecco's phosphate buffered saline (Oxoid). An individual ovary was then placed on a clean slide in one drop of 0.2 M sucrose made up in distilled water. The ovary was teased apart and the oocytes dispersed in the drop after removal of the ovarian debris. Between 250–400 oocytes were obtained per ovary. The slides were usually allowed to dry for 30 min, although when left overnight, quality was not impaired. They were then fixed for ten min in 4% paraformaldehyde (in 3.4% sucrose) and rinsed in 0.4% Photoflo (both fixative and Photoflo were adjusted to pH 8.5 with borate buffer). Staining was carried out by the rapid colloidal silver method of Howell and Black (1980).

To make a differential count of cell types present on successive days, preparations from the foetal ovaries were scanned systematically and all the meiotic stages seen were recorded (Table 1). Animals were first sacrificed on Day 13 of gestation, when oocyte development has reached the preleptotene stage (Borum, 1961; Peters et al., 1962; Crone et al., 1965) and continued until Day 1 postpartum ie day 20 in the Swiss strain. A minimum of 200 oocytes was analysed on each day. When cells in the pachytene stage appeared (day 14 onwards), a detailed search was made for evidence of pairing failure or irregularity, any univalent chromosomes (which stain more darkly with the silver technique) being recorded. Pachytene cells for detailed analysis were selected where the minimum of overlapping of the silver-stained elements (synaptonemal complexes, SC) was apparent (see part II, Speed and Chandley, in preparation). Length measurements of the pachytene elements were also made on different days of gestation in order to detect any possible changes in contractual state of the bivalents with gestational age. For this, photographic enlargements were prepared to a total magnification of 3,000×. Each SC was given an arbitrary number and

Table 1. Meiotic cell distribution in Swiss foetal oocytes prepared by surface spreading on successive gestational days (expressed as percentages)

Cell stage	Day of gestation							
	13	14	15	16	17	18	19	20
Pre leptotene	*100*	*78.0*	36.0	17.7	22.0	0.7	–	–
Leptotene	–	17.0	*36.7*	6.7	9.7	8.3	–	–
Zygotene	–	–	5.7	2.7	2.0	0.3	–	–
Late zygotene – early Pachytene	–	5.0	21.6	*69.3*	*47.0*	25.3	0.7	–
Pachytene	–	–	–	3.6	19.3	*53.7*	21.0	1.6
Diplotene	–	–	–	–	–	11.7	*42.0*	7.7
Dictyate	–	–	–	–	–	–	36.3	*90.7*
Total cells scored	200	200	300	300	300	300	300	300

its ends marked by a dot on the print. Measurements of the SCs on ten prints were made using the cursor of a Digiplan electronic measurer (Reichart-Jung). Each measurement was repeated on another day in order to minimise error. Relative lengths of SC's were expressed as a percentage of the total length of the genome.

Results

Description of Meiotic Stages

Prepleptotene. This is a cell stage which in air-dried preparations is extremely difficult to identify with certainty (Fig. 1a). When silver staining is applied however, the nucleus shows a characteristic morphology. The chromatin is diffuse and homogeneous in appearance, but contains a variable number of silver-stained bodies in a range of sizes and shapes. Some of the larger bodies may be nucleolar in nature while the small ones perhaps represent the centromeric ends of chromosomes. Sometimes these can be seen in pairs (Fig. 1b).

Leptotene. At this stage in air-dried preparations, chromosomal condensation has occurred but the chromosomal elements still appear unpaired (Fig. 1c). With surface spreading however, a fine network of silver-positive threads (lateral elements) make their appearance for the first time, and these confirm the absence of synapsis. The small stained bodies seen at preleptotene have now largely disappeared but the larger nucleolar bodies persist (Fig. 1d).

Zygotene. Evidence of pairing is now seen in cells prepared either by air-drying or surface-spreading (Fig. 1e, f). It is characteristic of this stage that synapsis is incomplete and in particular many chromosome ends remain unpaired. The paired lateral elements of the SC's however are characteristically aligned in parallel fashion. The nucleoli can now be clearly identified and are variable in number. Examination of the nucleolar bivalents shows that the ends of the bivalents remaining unpaired at this time are the centromeric ends. Delayed pairing has also been reported for the centromeric end of the acrocentric (nucleolar) bivalents in human spermatocytes examined at zygotene (Holm et al., 1979). Even amongst the non-nucleolar bivalents the presence of small discrete silver-stained bodies which characterize the centromeres (kinetochores) (Moses, 1977; Dresser and Moses, 1979) can sometimes be seen and, again, it is clear that these are the ends of the bivalents which remain at this time unsynapsed.

Pachytene. The advantages of surface-spreading over air-drying are most obvious at this stage. Air-dried preparations show an obviously fully paired meiotic complement (Fig. 2a), but only in exceptional cells can numbers of synapsed bivalents be accurately counted or involvement with nucleolar material determined. With surface spreading however, it is immediately possible, not only to discern such detail, but also to distinguish late zygotene – early pachytene (LZP) from full pachytene. In the former, the synaptonemal complexes are almost fully formed but in some bivalents the centromeric ends still remain unpaired (Fig. 2b). At pachytene, the SC's are complete, including the kineto-

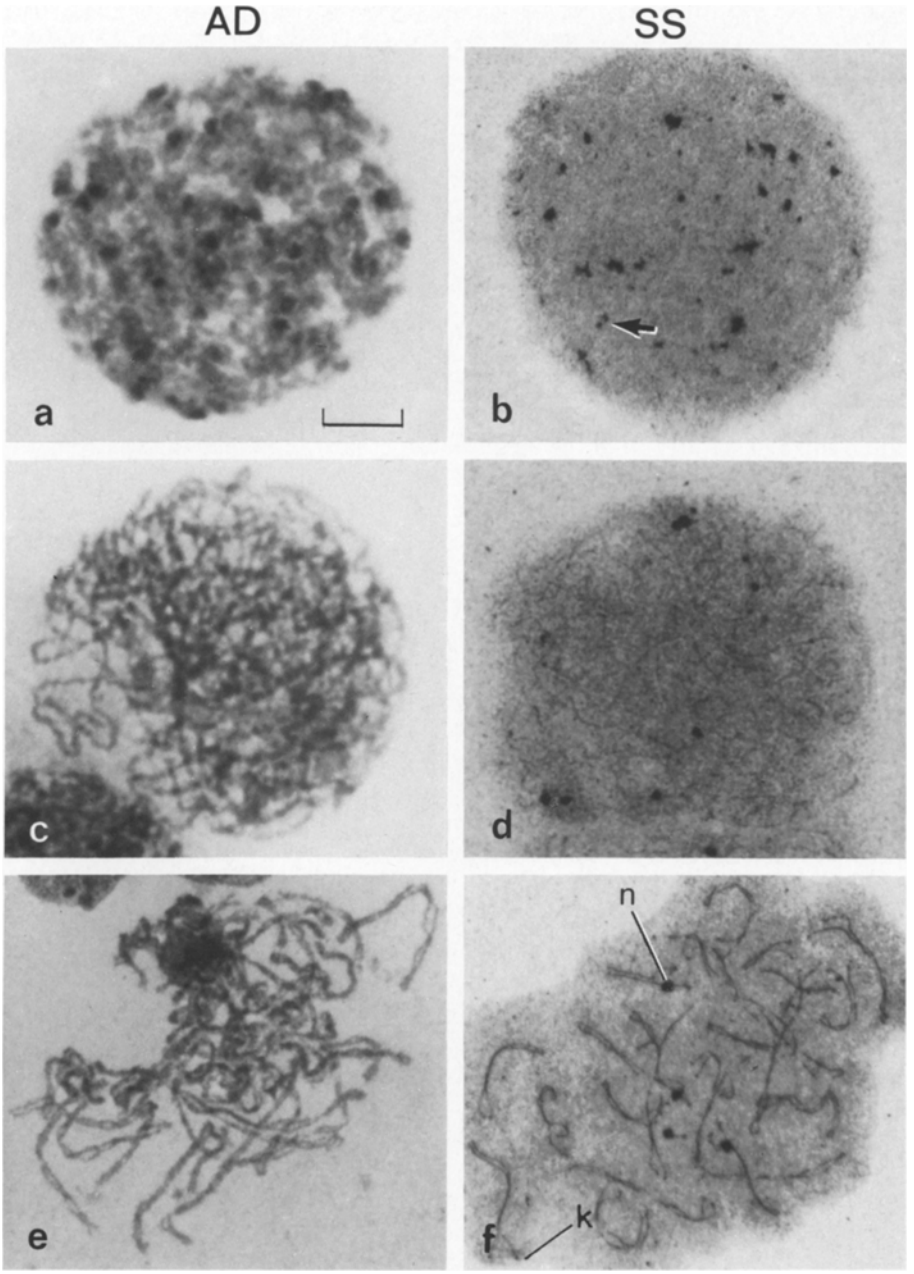

Fig. 1. Early stages of meiosis in mouse foetal ovaries. Air-dried (*AD*) giemsa-stained cells, compared with surface-spread (*SS*) silver-stained preparations. **a–b** Preleptotene. *Arrow* indicates paired centromeres. **c–d** Leptotene. **e–f** Zygotene. *n* nucleolus *k* kinetochore. Bar represents 10 μm

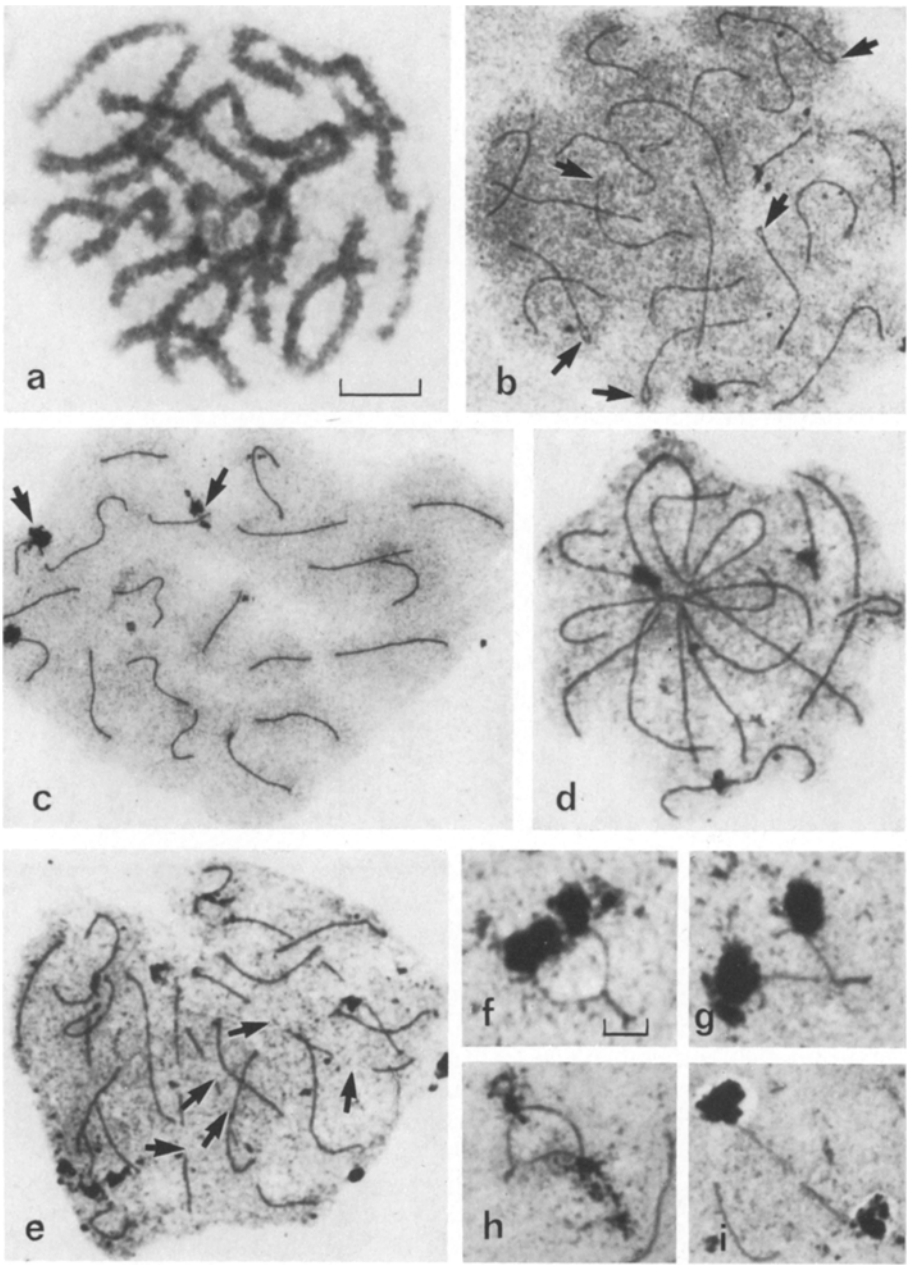

Fig. 2. Pachytene stages of meiosis in mouse foetal ovaries. **a** Pachytene (AD). **b** Late zygotene-early pachytene (SS). Arrows indicate unpaired centromeric ends of synaptonemal complexes. **c** Pachytene (SS). Synaptonemal complexes have contracted in length, and may bear one or two nucleoli (arrows). **d** Polarised synaptonemal complexes (SS) in a "bouquet-like" stage. **e** Pachytene cell (SS) showing gaps in the synaptonemal complexes. **f–i** Influence of nucleoli on the smallest synaptonemal complex (No. 19) at the end of pachytene. Separation of the lateral elements ranges from partial (**f–h**) to complete (**i**). Bar represents 10 μm for a–e, or 1 μm for f–i

chore region, and the bivalents become more contracted (Fig. 2c). The mean length of the SC's for the whole complement has been reduced by 36.6% from 271 μm at LZP, to 172 μm at pachytene. Such contraction during pachytene has also been observed in spermatocytes of different species (Moses et al., 1977; Tres, 1977; Jones and Wallace, 1980).

In about 0.5 per cent of all pachytene cells, the SC's are polarised (Fig. 2d). The majority (66%) of cells exhibiting such a "bouquet-like" stage, occurred on day 16. In the mouse oocyte however, when examination has been made in squash preparations, a "bouquet" stage appears not to be well developed (Bakken and McClanahan, 1978). About 25 per cent of the pachytene cells seen over days 16–19 of gestation exhibited non-staining gaps in the SC's (Fig. 2e).

Perhaps our most interesting observation however was made in the pachytene oocytes analysed on days 18 and 19, the last two days of gestation. Out of 555 pachytene cells examined over these two days, 65 (11.7%) showed clear desynapsis in the smallest bivalent of the genome (No. 19) while the rest of the complement remained fully paired. This bivalent bears a prominent nucleolar organiser region (NOR) and as the NOR material dispersed at the end of pachytene (see later), so the lateral elements of the SC of the bivalent No 19 appeared to be pulled apart, starting at the site of nucleolar attachment (Fig. 2f–h) and progressing until, in a few cells, the half-bivalents were completely desynapsed (Fig. 2i). The same observation was made at pachytene in oocytes of the CBA strain examined on the last two days of gestation and in that strain too, it occurred with a similar frequency (see Part II, Speed and Chandley, in preparation).

With regard to the nucleoli in general, these remained prominent during pachytene although becoming fewer in number as the stage progressed.

Diplotene. Characterised by the separation of homologues, this stage is a difficult one to interpret by either of the preparative techniques. It is also very infrequent on the air-dried slides. A cell from an air-dried preparation in what we would term the "diplotene" stage is shown in Fig. 3a. The bivalents are generally unclear in appearance and, in our opinion, the accurate counting of chiasmata in them would be impossible.

In the surface-spread preparations, cells are seen on the late days of gestation (days 18–19) which we would also define as diplotenes. They show lightly stained chromosomal elements which are now elongated and desynapsed (Fig. 3b). Many bivalents display a long interstitial segment of desynapsed chromosome giving a balloon-like appearance to the bivalent, which however remains paired at the two ends. By contrast with cells in zygotene, a stage which in the male may perhaps be confused with diplotene, but which in the ovary is found on a much earlier day of gestation, the nucleoli at diplotene are less discrete, irregular in shape and granular in appearance. As diplotene cells progress into the dictyate stage, the chromosomal elements become even less distinct, while the nucleoli from into a small number of large granular bodies.

Dictyate. The oocyte nuclei are now very large and the chromosomes decondensed in air-dried preparations (Fig. 3c). Surface-spread preparations show

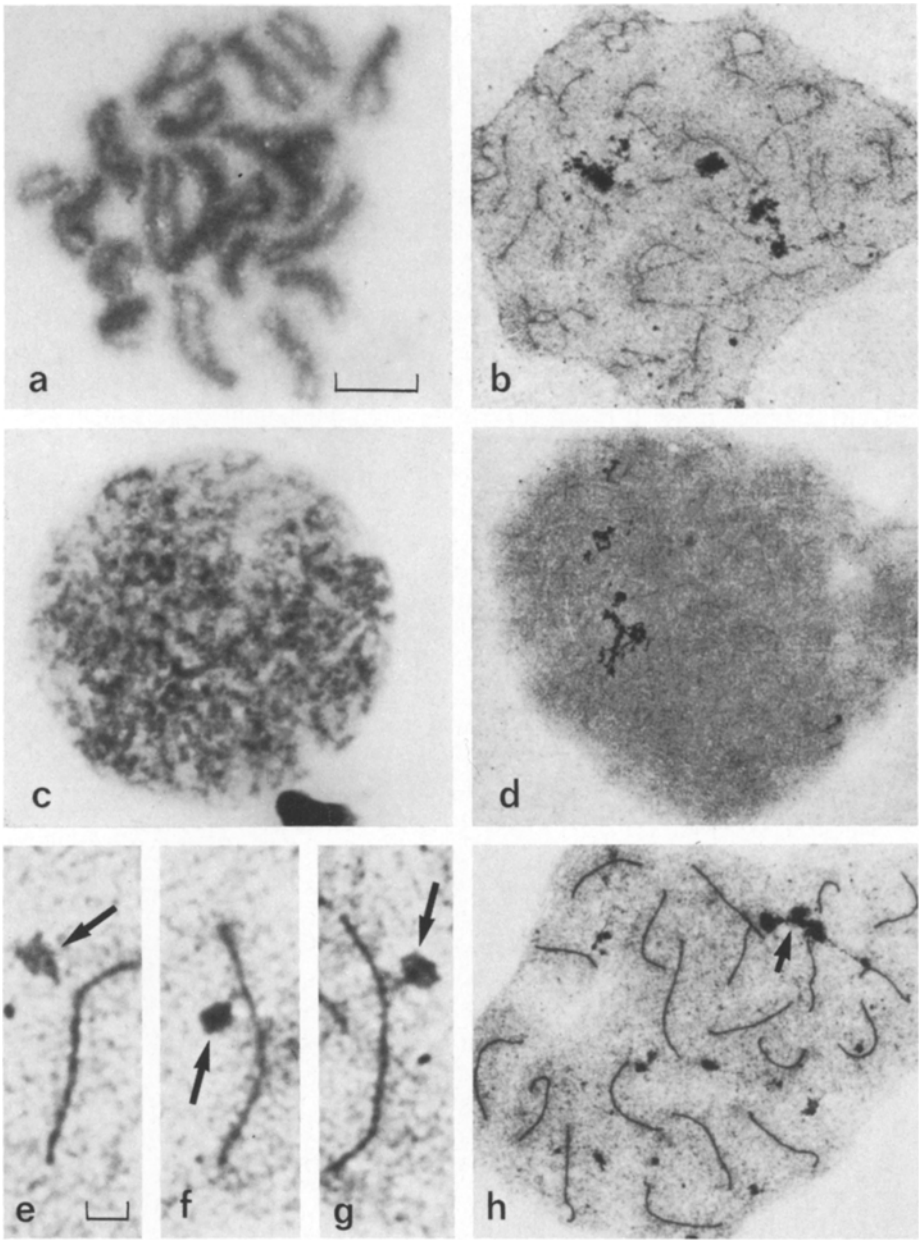

Fig. 3. Late stages of meiosis in mouse foetal ovaries. **a** Diplotene (AD) **b** Diplotene (SS). **c** Dictyate (AD). **d** Dictyate (SS). **e–g** Interstitial nucleolus seen on one medium-length synaptonemal complex at pachytene (SS). **h** Arrow indicates nucleolar association, as seen in 1% of pachytene cells. Bar represents 10 μm for a–d and h, or 1 μm for e–g

little or no evidence of any remaining silver-stained chromosomal elements. The stage is characterised however by several very prominent granular nucleoli which appear to be fragmenting against a homogeneous background of chromatin (Fig. 3d).

Meiotic Progression by Day of Gestation. A differential count of the meiotic stages encountered on successive days of gestation in surface-spread preparations is given in the Table. On day 13, all oocytes are in preleptotene. By day 14, some leptotenes and a few early pachytenes have appeared. On day 15, increasing numbers of LZP cells are seen but the majority cell types are still preleptotene and leptotene. Day 16 is the peak day for LZP cells. Borum (1961) also found this in her squash preparations and described a "burst" of pachytenes on this day. She also noted that some pachytene cells at this time appeared degenerate, and in our surface-spread preparations, the non-staining gaps which were observed in the SC's of about 25 per cent of pachytene cells over days 16–19 could possibly have been a feature associated with degeneration. Alternatively, they may be breaks caused by rupture and spreading of the nucleus. Gaps have also been observed in the SC's of grasshopper (*Melanoplus differentialis*) by Solari and Counce (1977) and in human spermatocytes (Holm et al., 1979; Solari, 1980) between the leptotene and pachytene stages. Solari and Counce (1977) however, found that normal length values for the grasshopper X-chromosome axis were only attained if such gaps were included in measurement data. They suggested that masking was probably responsible for such gaps in the X axis as opposed to mechanical stretching brought about by preparative technique.

Day 17 also showed most oocytes to be in the LZP stage. By day 18 the majority had reached pachytene but a few diplotenes had now appeared, again a finding consistent with Borum's (1961) observations. On day 19, the dictyate stage had been reached by one-third of the oocytes but the majority were still in diplotene and a few still in pachytene. Birth usually occurs by the morning of day 20 in the Swiss strain and at this time almost all cells were in dictyate (90.7%). A few pachytenes and diplotenes however still remained.

Nucleolar Number and Activity. Nucleoli, which are visible at all stages of prophase in silver-stained cells, decreased in number but increased in size as cells progressed from preleptotene to pachytene. Preloptotene oocytes had an average of 6.80 per cell while at pachytene the number had declined to 2.57 per cell. By the dictyate stage only one or two large but fragmenting nucleoli remained (Fig. 3d).

Numbers of nucleoli are strain dependent in the mouse, and up to six chromosome pairs may bears NORs (Elsevier and Ruddle, 1975; Winking et al., 1980). Mirre et al. (1980) have shown that on the nucleolar chromosomes of the mouse oocyte both nucleolar organisers may be active giving rise to independent nucleoli, thus allowing a possible maximum of twelve nucleoli per cell.

At pachytene in our silver-stained preparations, while some nucleolar bivalents exhibited two active sites, others showed only one (Fig. 2c). An interstitially

located silver-stained body, resembling a small nucleolus, was seen in about 20 per cent of pachytene cells on Day 18 (Fig. 3e–g). This was somewhat paler staining than the terminally located nucleoli and attempts, by measurement, to determine which bivalent carried it were unsuccessful. This interstitial body first appeared in a few pachytenes on day 17 and grew in size as pachytene proceeded. It had disappeared by the diplotene stage. Interstitial nucleoli have previously been reported in human pachytene oocytes and spermatocytes prepared by air-drying (Stahl and Luciani, 1972).

Nucleolar associations were seen only infrequently in the surface-spread preparations: Only 12 out of 1144 pachytene oocytes examined showed such associations (Fig. 3h).

Discussion

The findings in surface-spread preparations of Swiss mouse oocytes are entirely consistent with earlier studies into the meiotic progression of oocytes in five other strains of the mouse based on squash preparations and histological sections (Borum 1961). A certain degree of asynchrony seems to exist over days 13–19 of gestation, but remarkably, by day 1 post-partum, over 90 per cent of oocytes are in the dictyate stage. How this "catching-up" is achieved is hard to say, but it is possible that oocytes which lag behind in development become atretic and degenerate. Borum (1961) and other authors have reported on oocyte degeneration in the pachytene stage and the pachytene oocytes which we observed to have non-staining gaps, and which made up about one quarter of all pachytene oocytes seen on the slides over days 16–19 could perhaps have been destined to become atretic. Since the period over which mouse oocytes enter meiosis extends over about 3 days, (according to the ^{3}H-labelling studies of Peters et al., 1962), it would seem that some mechanism must operate to account for the fact that the vast majority of oocytes observed on day 1 post-partum are in the dictyate stage, only one-third having reached that stage by day 19 of gestation. If delay in development means that any particular oocyte is destined to become atretic, then the concept of a "production line" in the foetal ovary becomes meaningless. Indeed, some authors (Baker, 1972) have already questioned the existance of a production line in the mouse ovary because of the short time over which meiotic prophase extends (Crone et al., 1965), and because of the relative degree of synchrony observed in oocyte maturation.

The interesting observation of desynapsis in the nucleolar bearing bivalent No. 19 at the very end of pachytene is, to our knowledge, something which has not been reported before. It would appear that this small bivalent commences desynapsis for diplotene in advance of all other bivalents in the complement. In a few cells, the process has advanced so far that desynapsis is complete and two No. 19 univalents are present in the late pachytene cells. This same phenomenon is observed in the CBA strain and also appears to involve chromosome 19, not any other of the nucleolar bearing chromosomes. The possible consequences for subsequent non-disjunction of this small chromosome pair in the adult female will be discussed in Part II of the paper. The finding that 0.5% of the pachytene cells show a clear bouquet stage i.e. after the

process of pairing is complete, together with the previous findings of occasional cells with a "bouquet-like" appearance (Darlington, 1965) points to a possibility that a bouquet stage may occur in all cells at some time during pachytene, but that the bouquet is disrupted as a consequence of preparative technique.

The usefulness of the surface-spreading method for oocyte analysis at the light microscope level is, we believe, well illustrated by the preparations we have obtained in the mouse. Investigations are currently underway also to examine human foetal oocytes from the ovaries of aborted conceptuses by the same method. The chromosomal detail in that species too outweighs anything which can be obtained by air-drying or squash techniques.

Acknowledgements. The author wishes to thank both Dr. A.C. Chandley and Professor H.J. Evans for helpful discussion of the manuscript.

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Received March 25, 1982 / Accepted by H. Macgregor