Submicroscopic Organization of the Nucleus during Spermiogenesis in the Grasshopper.* By C. M. S. Dass‡, and Hans Ris. (*From the Department of Zoology, University of Wisconsin, Madison, Wisconsin.*)§

Recent electron microscope studies of nuclei and chromosomes have shown that a microfibril from 100 to 200 A thick is the basic morphological unit of chromosomes. Chromosome fibrils of this dimension have been reported in the salivary gland chromosomes of *Drosophila virilis* (20, 21), in salivary chromosomes of *D. melanogaster* (9), in leptotene, pachytene, and metaphase chromosomes of *Lilium* (13–16), in carp erythrocyte nuclei (22), in the somatic interphase nuclei of *Lilium*, *Allium*, and *Tradescantia*, in the nuclei of liver, pancreas, and testis tissues of the rat and in plasma cells, monocytes, and lymphocytes of mice (4), and in the staminal hairs of Tradescantia (12). We know that in somatic nuclei the chromosomes contain not only deoxyribonucleic acid (DNA) and histones, but also non-histone protein and ribonucleic acid (RNA) (3). During spermiogenesis, however, profound changes take place in the chemistry of the nucleus. Histones are usually replaced by protamines and non-histone proteins are either greatly reduced or absent (7). Inasmuch as the sperm nucleus still carries genetic material, it would be of interest to know what changes in submicroscopic organization of chromosomes are associated with these modifications in chemical composition. In this preliminary note, we wish to report some observations on nuclear structure during spermiogenesis in several species of grasshoppers.

Individual follicles from testes of the grasshoppers, *Chortophaga viridifasciata* (De Geer), *Chorthippus curtipennis* (Harris), and *Romalea microptera* (Beauvois) were dissected in insect Ringer solution and fixed immediately in cold (5°C.) 2 per cent OsO_4 buffered with acetate-veronal to pH 7.5, dehydrated, and embedded in 1:4 mixture of methyl and *n*-butyl methacrylate with benzoyl peroxide as catalyst. Thin sections ranging from 25 to 50 mμ thickness were cut with a Porter-Blum microtome. Electron micrographs were taken on Ilford N.40 process plates with an RCA electron microscope type EMU-2.[1] An objective aperture 50 μ in diameter was used.

The nucleus in early spermatids has the typical organization of interphase. Sections of chromosome fibrils appear to be 200 to 250 A in thickness and seem to consist of two subunits about 100 A thick each. At this stage, in addition to chromosome fibrils, the nuclear space is filled with nucleoplasmic material (Figs. 1 and 8). During the process of maturation, the spermatid nucleus progressively elongates. Thus length and cross-sectional diameter of the nucleus can be used as independent criteria for the seriation of the stages in sperm development. As spermiogenesis advances, the arrangement of chromosome fibrils becomes more regular as they orientate more or less parallel to the long axis of the elongating sperm nucleus. At this stage individual microfibrils are 100 to 150 A thick and in places appear to separate into finer fibrils of about 40 A, and simultaneously the nucleoplasmic material becomes greatly reduced (Figs. 2 and 9). The 40 A fibrils increase in number apparently at the expense of the 100 A thick microfibrils. With further elongation of the spermatid nucleus, the 40 A fibrils seem to join side by side to form membranes and these run in an irregular undulating manner in the nuclear space. They are seen to be arranged in pairs and to form an anastomosing network (Figs. 3, 4, and 10). The spaces between the chromosomal membranes are free of any visible structures. Further elongation of the nucleus is accompanied by a gradual narrowing of these spaces. The membranes become stretched and present in oblique section a regular honeycomb-like pattern (Fig. 5). Finally, in the mature sperm nucleus the spaces between the membranes disappear, and the nuclear material becomes uniformly electron dense (Figs. 6 and 7).

Preliminary cytochemical studies[2] on the same material indicate that the changeover from histone to protamine, and the disappearance of

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[2] For methods see reference 2.

non-histone proteins occur at the time when the 40 A fibrils become discernible and the intranuclear membranes are formed. That the dimensions of the nucleoprotein in sperm are related to its composition is also indicated by the work of Bernstein and Mazia (5). They reported that the nucleoprotein fibers isolated from mature sperm were 200 to 300 A thick, and their analysis revealed 25 to 27 per cent non-basic protein. It is interesting to note that the thickness of these isolated fibrils corresponds to the 200 A fibrils found in somatic interphase. Furthermore, in thin sections cut through sea urchin sperm nuclei 100 A thick granules have been observed (1) which probably represent sections of 100 A fibrils similar to those found in interphase nuclei.

Membranous structures in the nucleus of spermatids have been reported by Gibbons and Bradfield (10) in another species of grasshopper, *Melanoplus femur-rubrum*. They found "osmiophilic membranes" 100 A thick, oriented approximately along the axis of the head. Similar appearances were demonstrated by Grassé *et al.* (11) in the garden snail, *Helix pomatia*, but were interpreted to show a gradual straightening of the much twisted chromonemata 70 to 80 A thick, until in the mature sperm, they are all orientated in the long axis of the sperm head. From their beautiful electron micrographs we can conclude, however, that the changes in the nuclear organization is rather similar to what occurs in the grasshoppers. It is most unlikely that twisted fibers 100 A thick would lie in a single plane of section for such great lengths. In other words, the "fibers" of Grassé *et al.* are probably profiles of membranes.[3] Many stages in this remarkable metamorphosis of the nucleus in the snail show a striking similarity to what we find in the grasshoppers, and the membranous organization is especially clear in the electron micrographs shown in their Figs. 1 to 6, Plate X; Figs. 3 to 5, Plate XI; Fig. 1, Plate XII. It is interesting to note that nucleoprotamine extracted in 10 per cent NaCl and precipitated by dilution can organize into membranes (8).

Not all animals, however, show such a membranous structure of the nucleus during spermiogenesis. In the spermatid of *Bufo arenarum* the nuclear material is distributed as dense granules of various sizes (6). In the sparrow, *Passer montanus* saturatus, the nuclear material in the early spermatid is distributed similarly as dense masses 20 to 45 mμ diameter, which in turn are composed of microfibrils 100 A thick (19, 23). In late spermatids of the cockroach, *Periplanata americana*, the chromosome fibrils are aggregated into granules as in vertebrates; the smallest fibrils are about 40 A thick (17). Our observations indicate that in the sperm nuclei in the species studied the basic morphological unit is a fibril 40 A thick. In some sperm these fibrils associate into membranes, while in others they form dense granular masses.

[3] This conclusion has also been expressed by Rebhun, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 509.

SUMMARY

A study of the submicroscopic organization of the nucleus during spermiogenesis in grasshoppers has revealed that in early spermatids the chromosomes seem to be composed of microfibrils 200 to 250 A thick, which in turn are made of two subunits 100 A each. With the disappearance of the non-basic proteins at the beginning of elongation of the spermatid nucleus, the 100 A microfibrils resolve further into finer fibrils about 40 A thick. These fibrils associate into a network of membranes which finally become closely packed into a homogeneous mass in the mature sperm.

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Explanation of Plates

Plate 61

Fig. 1. *Chortophaga viridifasciata.* Electron micrograph showing the nucleus of an early spermatid. Chromosomal microfibrils (enclosed in circles) 200 A thick can be seen distributed in the finely fibrillar nucleoplasm. See enlargement in Fig. 8. Magnification, 35,000.

Fig. 2. *Chorthippus curtipennis.* Electron micrograph showing the elongating sperm nucleus. The fibrils 100 A thick (indicated by thin arrows) are seen orientated parallel to the long axis of the nucleus. Finer fibrils of 40 A thickness (indicated by thick arrow) can also be seen. See Fig. 9 for enlargement. Magnification, 44,000.

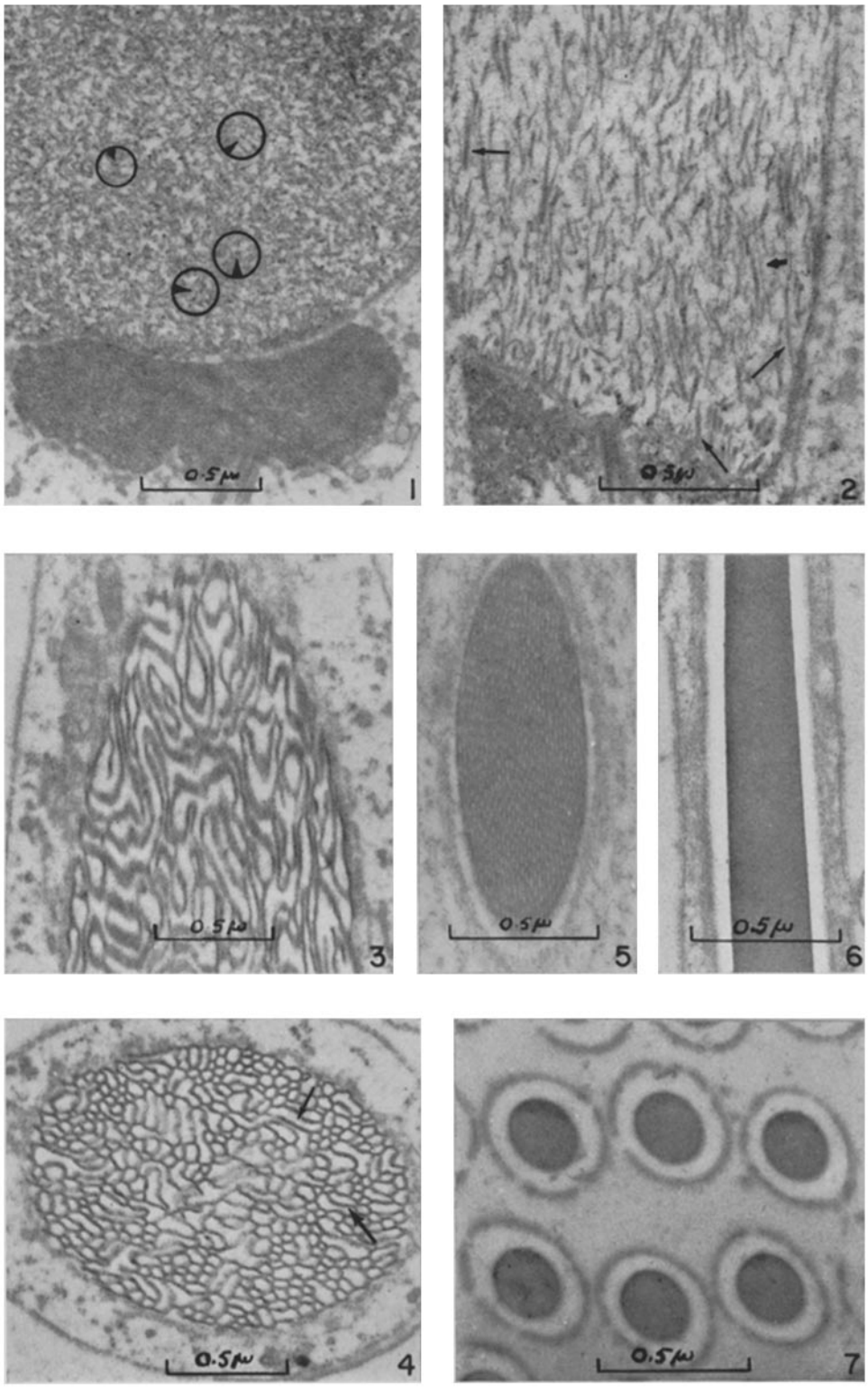
Fig. 3. *C. viridifasciata.* Electron micrograph of an oblique section through the nucleus at a stage later than Fig. 2, showing the membranous organization of chromosome microfibrils. Magnification, 35,000.

Fig. 4. *C. viridifasciata.* Electron micrograph of a near cross-section of the nucleus at a stage similar to Fig. 3. The arrow indicates the closely associated membranes. See Fig. 10 for enlargement. Magnification, 35,000.

Fig. 5. *C. viridifasciata.* Electron micrograph of an oblique section through the nucleus of an almost fully formed sperm. The orderly arrangement of membranes to form honeycomb pattern is seen. Magnification, 44,000.

Fig. 6. *C. viridifasciata.* Electron micrograph of a longitudinal section through the nucleus of a fully formed sperm. The nucleus appears homogeneously dense, presumably as a result of the tight packing of chromosomal membranes. Magnification, 44,000.

Fig. 7. *C. viridifasciata.* Electron micrograph of a near cross-section of several mature sperms. Magnification, 44,000.



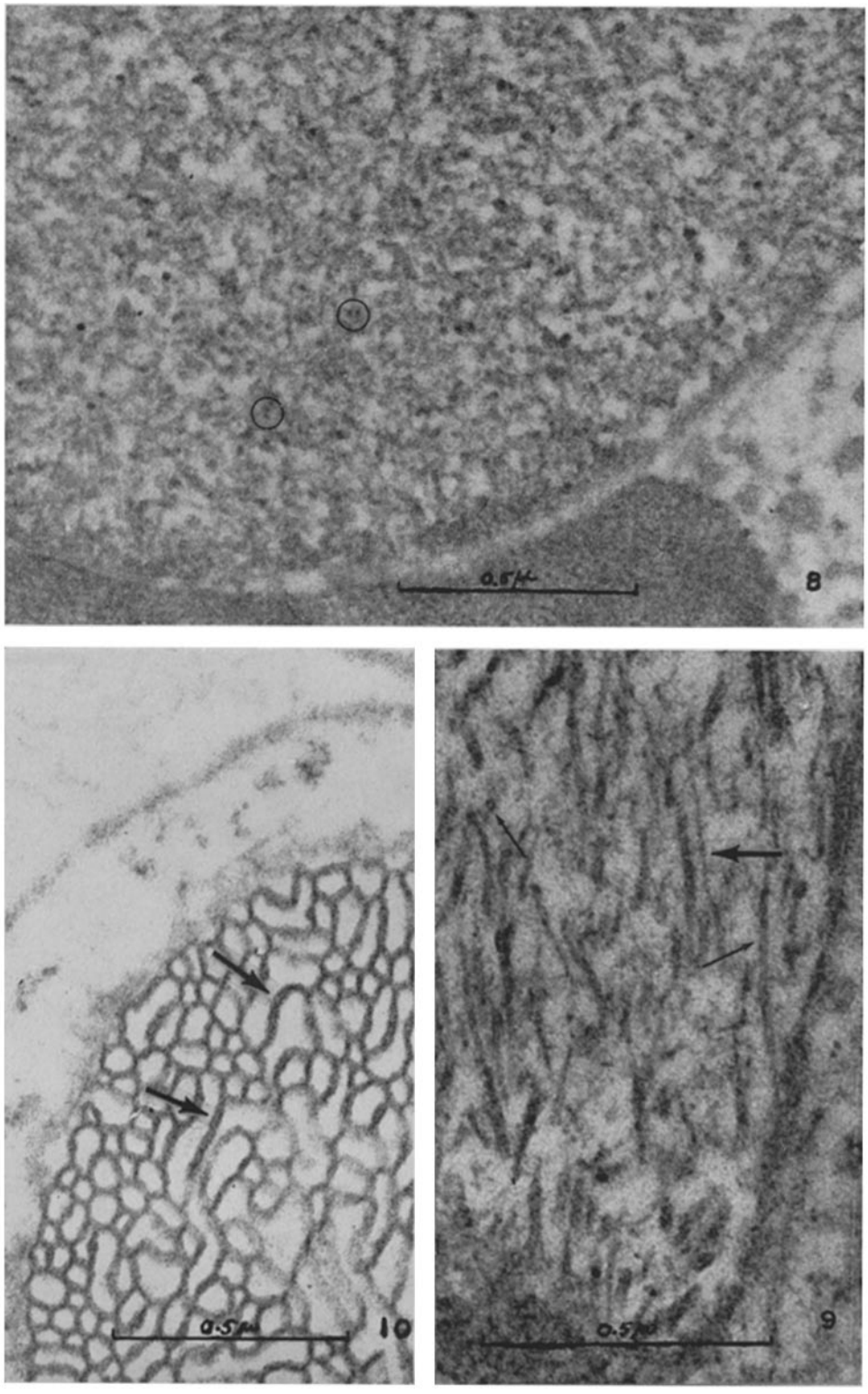
(Dass and Ris: Nucleus during spermiogenesis)

PLATE 62

FIG. 8. *C. viridifasciata.* Electron micrograph same as in Fig. 1, enlarged to show the 200 A microfibrils (enclosed in circles). Magnification, 69,000.

FIG. 9. *C. curtipennis.* Electron micrograph same as in Fig. 2, enlarged to show 100 A fibrils indicated by thin arrows and also finer fibrils of 40 A thickness (thick arrow). Magnification, 84,000.

FIG. 10. *C. viridifasciata.* Electron micrograph same as in Fig. 4, enlarged to show the close association of two membranes in their undulating course in the nuclear space (arrows). Magnification, 69,000.



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