Discussion. Data in the literature are consonant with the present studies. The creatinine clearance of non-hydropenic dogs has been shown to be unaffected by the infusion of hypertonic glucose(3) or urea(4) solutions. Other studies(5) showed similar results with sucrose and mannitol loading. The renal plasma flow was not determined in the experiments cited.

Summary. Osmotic diuresis produced by mannitol loading in hydropenic dogs does not significantly affect the glomerular filtration rate, renal plasma flow or filtration fraction.

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2. Rapoport, S., Brodsky, W. A., West, C. D., and Mackler, B., *Am. J. Physiol.*, 1949, v156, 433.

3. Shannon, J. A., *Am. J. Physiol.*, 1938, v122, 782.

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5. Cizek, L. J., and Holmes, J. H., *Am. J. Physiol.*, 1950, v160, 536.

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Electron Microscope Studies on Structure of Mitotic Figure.* (18026)

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The structure of the amphiaster has long been a subject of debate [Wilson(1) and Schrader(2).] The majority of evidence covered in these reviews indicates that the spindle is composed of fibers. The electron micrographs presented here also show the fibrillar structures in the spindle. The terminology here used in reference to the spindle components is that recommended by Schrader (2).

Material and methods. The material is from a crayfish testis, actively forming germ cells. It was fixed in Bouin's solution, embedded in a mixture of 60% paraffin (melting point, 70°C) and 40% beeswax and sectioned at 0.5μ with a Spencer No. 820 microtome equipped with a Spencer thin section adapter. The electron microscope used was an R.C.A. model E.M.U. equipped with an unbiased gun. The magnification of the figures may be determined from the micron scale appearing on each figure.

We wish to emphasize that since the cells here studied were fixed and dried the element of artifact must be kept in mind in analyzing the pictures.

Results and discussion. A portion of one-half of a longitudinal section of a metaphase spindle is shown in Fig. 1. The shape of the mitotic figure is typical of that seen in the usual preparations studied by the light microscope. The "chromosomal fibers" are about 1500-2500 Å in width. The fiber on the right side of the figure appears to be split, suggesting that the large spindle fibers in the crayfish are compound. Further evidence that the fibers are compound, *i.e.*, composed of many smaller fibers, is illustrated in Fig. 4, 5 and 8. The tangential section of the large "chromosomal fibers" in Fig. 4 clearly shows the cut ends of the smaller fibers within the compound larger ones. In addition, an apparent lateral stretching of the large "chromosomal fibers" has separated their small component fibers, in Fig. 5 and 8. The smaller fibers are about 300-600 Å in width. Some of the smaller fibers show irregular banding or beading which possibly is the result of irregular contraction at the time of fixation. Similar phenomena may also be

* We are indebted to the General Biological Supply House, Inc., Chicago, for kindly supplying us with the fixed crayfish testis.

1. Wilson, E. B., *The Cell in Development and Heredity*, p. 114, Macmillan Co., N.Y., 1928, 3rd edition.

2. Schrader, F., *Mitosis*, Columbia University Press, N.Y. 1944, p. 4.

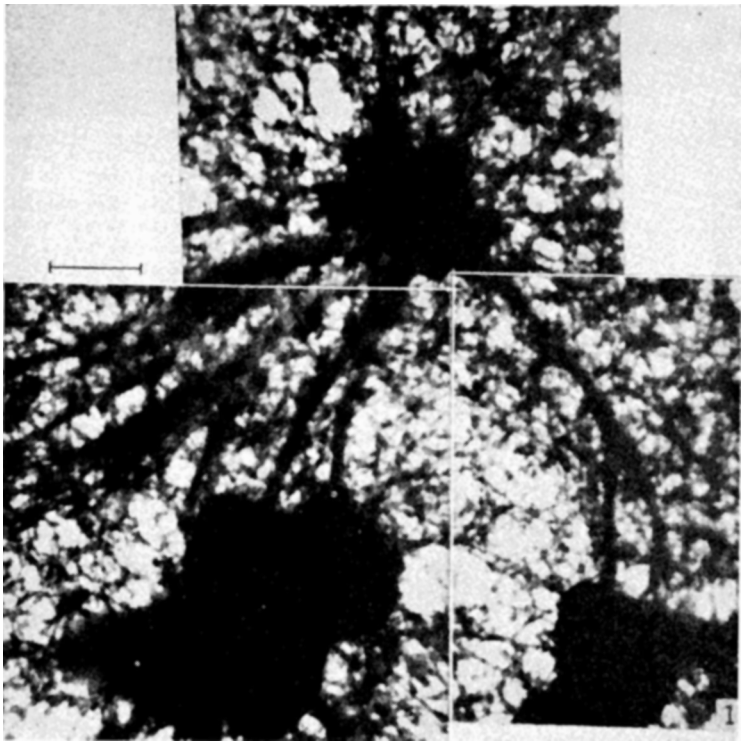

FIG. 1.
Portion of half of a longitudinal section of a metaphase spindle showing centrosome, chromosomes, "chromosomal fibers," and astral rays.

seen in the large "chromosomal fibers" of Fig. 2 and 3. In both cases the banding seems too irregular and too gross to represent periodic banding of their ultrastructure. We have not been able to identify clearly the "continuous fibers" (those extending from centrosome to centrosome) in this material. If present, they seem to be relatively small and few in number.

The centrioles are relatively dense and somewhat irregular in outline (Fig. 1, and 7). In Fig. 1 the section seems to have been through the middle of the centriole. Here short tongues of centriole material extend out along the base of the spindle fibers. In Fig. 7 the section through the centriole seems to have occurred near the periphery. This figure shows how the "chromosomal fibers" lose their orientation at the periphery of the centriole, and it indicates that the centriole is probably not a homogeneous body since varied darker and lighter areas appear within them.

Small astral rays are present about the periphery of the centriole (Fig. 1 and 7). They seem to be fibrous rather than hollow tubes. The asters in the crayfish are not as suitable for study of their structure as are those in the dividing whitefish blastula (unpublished).

A section through the interzonal region of an anaphase figure is shown in Fig. 6. Some orientation of small fibers appears between the chromosomes. These we are interpreting as the "interzonal fibers." Since the fibers here appear to be discontinuous in spots it is likely that a disorganization of them in this region is occurring. The net-like strands of protoplasm are seen extending between the larger longitudinally oriented "interzonal fibers."

The cytoplasm has the appearance of a fibrous sponge-like reticulum. This condition probably represents mainly the coagulated protein framework.

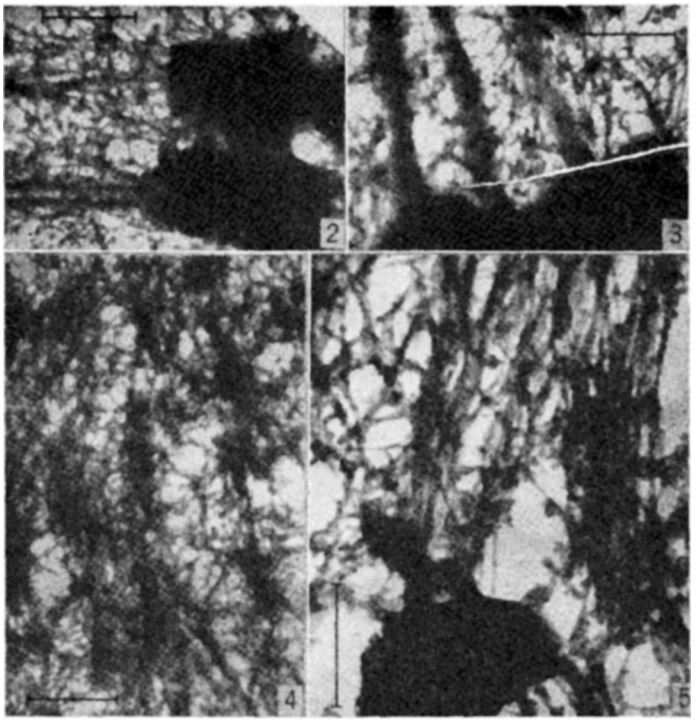

FIG. 2. Slightly laterally stretched "chromosomal fibers" with some banding in the lower fiber.

FIG. 3. Banding apparent in some "chromosomal fibers."

FIG. 4. Tangential section of "chromosomal fibers" showing cut ends of their smaller component fibers.

FIG. 5. Laterally distorted "chromosomal fibers."

As previously mentioned the method of preparation of this material has undoubtedly induced some degree of artifact. However, from the abundance of previous work on this subject with the light microscope (Schrader (2), it seems safe to conclude that for the most part the present figures of the centrosomes, "chromosomal fibers," "interzonal fibers" and astral rays represent at least roughly the condition in the living cell.

We were not able to differentiate between first spermatocyte and second spermatocyte divisions in our figures. However, from the work of Fasten(3) the appearance of the spindle under the light microscope is similar in both divisions.

Summary. The metaphase spindle of the maturation division in the crayfish testis seems to be composed largely of longitudinally oriented "chromosomal fibers" measuring about 1500-2500 Å in width. These fibers appear to be compound, *i.e.*, made up of smaller fibers of about 300-600 Å in width. Both the "chromosomal fibers" and their smaller fibrous components may at times show banding, which is possibly the result of irregular contraction during fixation. The centrioles appear relatively dense, are irregular in outline, and are probably not homogeneous bodies. The "chromosomal fibers" and astral rays lose their usual orientation at the periphery of the centriole. "Interzonal fibers" are relatively small in the late anaphase stages of the crayfish testis. Though "continuous fibers" were not clearly observed, we do not wish to claim that such fibers may not exist. Astral rays are composed of fibers, oriented as described in the past. The coagulated cytoplasm presents the appearance of a sponge-like reticulum, which is probably mostly a protein "framework," since

3. Fasten, N. I., *J. Morph.*, 1914, v25, 587.

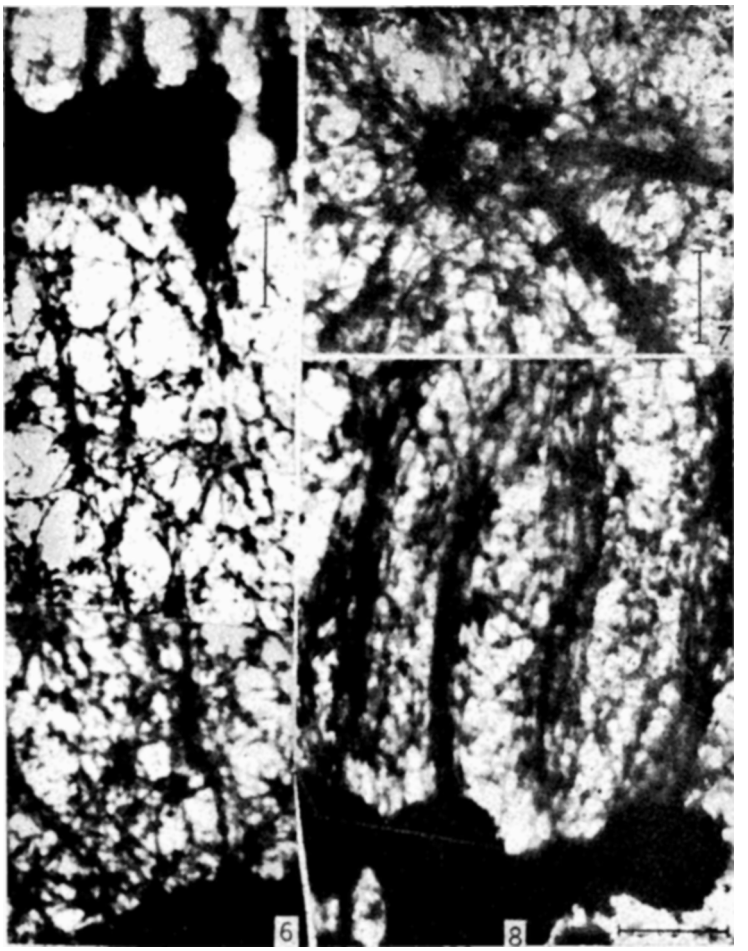

FIG. 6. Interzonal region of anaphase, with some orientation of "interzonal fibers." The lower print which is slightly out of focus does not exactly unite with the one above.

FIG. 7. Section through portion of a centrosome.

FIG. 8. Laterally stretched "chromosomal fibers" illustrating their compound nature.

much of the other materials had been removed by the method of preparation.

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