Preliminary Investigations of Chromosomes and Genes With the Electron Microscope

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A RELATIVELY SIMPLE METHOD of cutting and mounting sections sufficiently thin for effective use with the electron microscope was recently described by the present authors (*Proc. Soc. exp. Biol. Med.*, 1948, **67**, 470). Section 0.2 μ thick were described originally, but with practice and minor improvements, 0.1-μ sections often have been obtained. We have started to apply this technique in a study of chromosome structure. Work has been initiated with the salivary glands of *Drosophila melanogaster*, fixed in the conventional manner by removing the glands directly within an acetocarmine mixture. This mode of fixation proved to be rather better than we had anticipated, even at the highest magnifications.

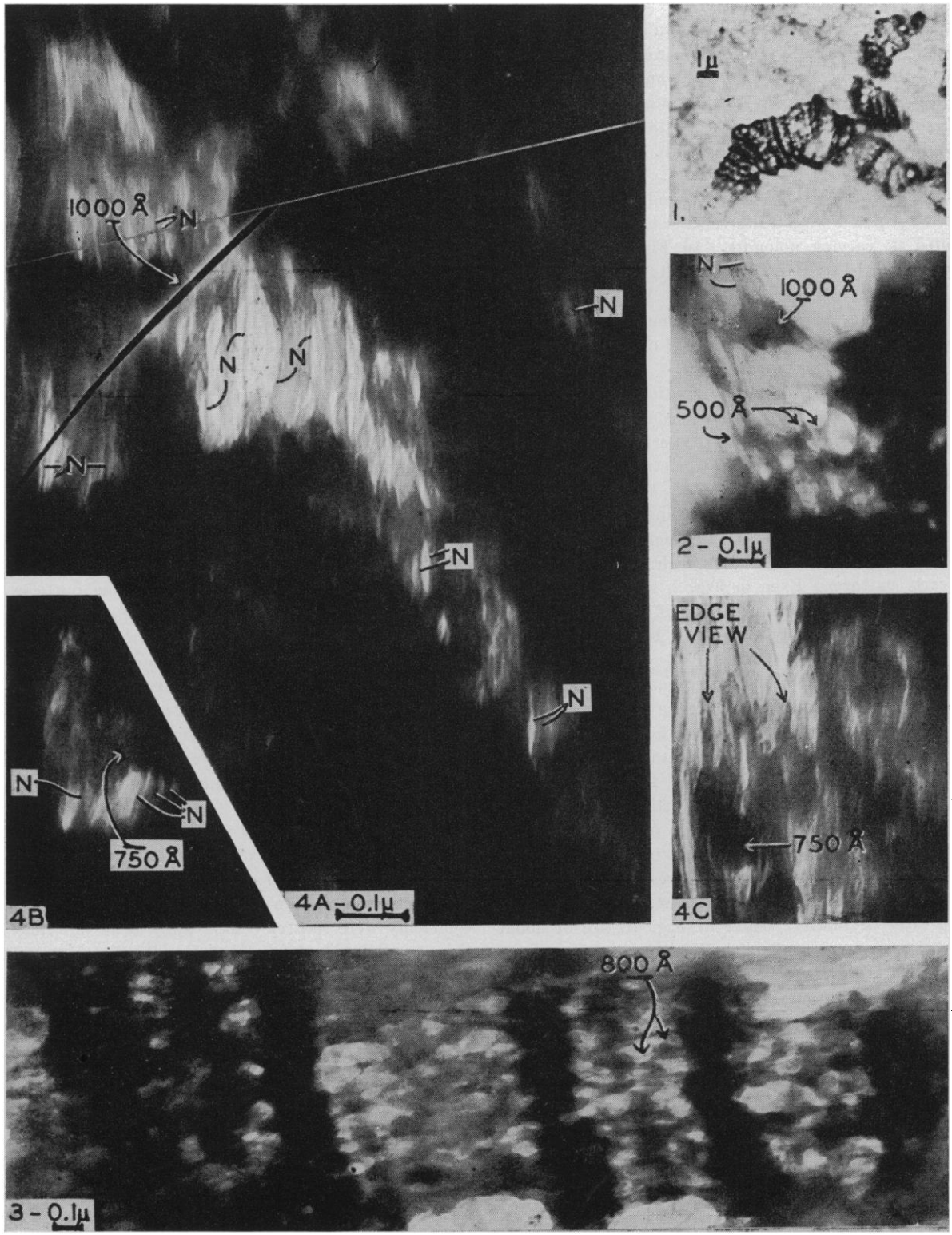
Before proceeding, it may be well to point out the known salient features of salivary chromosomes for those who are not familiar with their highly specialized organization. These are not ordinary chromosomes with single chromomere granules spaced along a single chromonemal thread. Instead, each primordial chromosome has reduplicated itself many times over. The end-product is a giant multiple chromosome made up of hundreds, if not thousands, of identical units.

Work with low magnifications disclosed particularly that the chromomere bands were very dense—far denser than any other cellular constituent. One has only to glance at Fig. 1 to be impressed with the difference between the substance of the chromomeres and the nucleoplasm. Since the electron image is a function of atomic and molecular density, it is possible to state definitely that chromomeres cannot have much water within them. The great density of the chromomeres has, in fact, been a major source of difficulty in attempts to employ high magnifications to resolve their structure. Excellent detail could be seen in any portion of a 0.1-μ section except within chromomeres. The latter have been visualized effectively only when their thickness was substantially less than 0.1 μ. This has occurred at the edges of sectioned chromosomes where their substance tapered (as in Fig. 2), or when sections had pulled apart somewhat to produce particularly thin areas (as in Fig. 4). Under those circumstances chromomeres have been resolved into their unit particles.

It will be convenient to begin our detailed discussion with Fig. 3, which shows a chromosome at moderate magnification for the electron microscope. The general relations show well, although fine detail is lacking. Chromomere granules arranged in bands are readily visible, the larger ones, however, being opaque to the electron beam. The lighter bands contain smaller granules usually aranged more diffusely. In one region, isolated 800-A particles are visible. The matrix is coarsely vacuolated—undoubtedly a fixation artifact, for the proteins of any greatly hydrated portion of a cell always are precipitated as a fibrous net. Between the vacuoles a mesh of relatively heavy fibers exists, too large and irregularly arranged to be identifiable directly with chromonemata. This figure shows most of the relations (and artifacts) that can be seen readily. One point that can be established definitely at this magnification is the fact that no limiting membrane can be found at the surface of the chromosome.

Under circumstances outlined earlier, detail was seen within chromomeres, but micrographs showing this clearly have been rarities. The remaining discussion will be based upon about a dozen pictures, although many more suggest and confirm the relations to be considered. Figs. 2 and 4 have been chosen to illustrate the unit particles of chromomeres, but some discussion of their general relations seems necessary. Fig. 2 was from the edge of a chromosome. Three completely dense chromomere bands can be seen to the right. To the left, 500-A particles in isolation, and also a small cluster of 1,000-A particles, can be seen. The larger particles were quite obviously related to a much heavier band than were the smaller ones.

Figs. 4, A, B, and C, are the most revealing electron micrographs obtained so far. The original micrographs have a resolution of at least 30 A, although detail is lost in reproduction. All are of the same preparation, but unfortunately, the plane of section was quite oblique to the long axis of the chromosome. The individual bands tend to be shingled, one overlapping the next. The course of the bands is nearly horizontal, as can be seen in 4B and at the upper left of 4A. The section was actually rather thick, but clefts opened to disclose thin areas.



Electron micrographs of *Drosophila* salivary chromosomes: Fig. 1, × 3,600; Fig. 2, × 76,000; Fig. 3, × 49,000; Fig. 4, × 120,000. Chromonemata are designated by N. Particle lengths are expressed in Angstrom units.

In Fig. 4, wherever one sees detail in the chromomere bands, one gets the impression of spindle-shaped particles. In this and other micrographs, whenever the spindles showed to good advantage, they had a uniform density across their width, indicating that the particles were flat plates. If they had had circular cross sections, the center axis would have been denser and blacker than the sides. Variations in the particle width within a single band further suggests that flat plates were being viewed obliquely, as though tilted in varying amounts. In Fig. 4C a few are thought to be seen on edge. The 1,000-A particles of Fig. 2 are of the same leaf shape. In fact, this seems to be very decidedly the most common type of unit particle which has been seen more or less clearly in a number of electron micrographs.

Particles having other configurations have been seen, however. These were globular in character rather than flattened in one plane. The type we have observed best and most often was asymmetrical. The 500-A particles of Fig. 2 are examples. We have good evidence of cigar-shaped particles, and others appeared to be essentially spherical. It should be stressed that within any one band the particles appeared to be nearly uniform. Even in Fig. 2, the small particles were presumably identical, the difference in appearance resulting simply from their being viewed from different angles.

The unit particles of the chromomere bands quite certainly were not of constant size from one band to another. Approximate lengths of some that can be measured most accurately are indicated on the figures. Because of the oblique section of Fig. 4, the lengths there may be deceptively low. Particles ranging up to almost 1,500 A have been seen in other micrographs. Particles of 500 A are the smallest that have been seen clearly, although there is some reason for believing that somewhat smaller ones exist. The particular particles of the left center of Fig. 4C are of about average size—approximately 750 A long and not more than 80 A thick. Although their width cannot be determined very accurately, it is probably about 250 A. The particle volume would therefore be of the order of $10^7 A^3$.

We have seen enough electron micrographs to realize that the grosser morphology of the bands is to some extent a reflection of the character of their unit particles. Particles of different shape apparently tend to aggregate in different manners. The most striking example is a very regular and uninterrupted alignment of cigar-shaped particles which has been observed to produce a band that would appear as a sharply defined, nongranular line in the light microscope. The leaf-shaped particles often were seen aggregated to form rather large granules, more or less fused with one another. This is the type of dense band visible in Fig. 3 and would definitely appear coarsely granular in the light microscope. Globular particles usually were observed only in small aggregates (Fig. 2 is an exception). The small granules so formed tended to exist in isolation and to be scattered. The light microscopist would see such bands as diffuse structures, very finely granular. No doubt other as yet undiscovered relations exist, and the time may come when these can be expressed more precisely.

There is an interesting corollary of these findings. Since the chromomeres are composed exclusively of the unit particles, and since theory (and our own observation) demands that every band contain the same number of units, the volume of stainable material in a band should be a function of particle size. If these particles can be identified as genes, gene size probably can be estimated with the light microscope.

Before leaving the discussion of the unit particles, it will be wise to consider the possibility of fixation artifact. The degree and kind of artifact cannot be established with certainty without much further work employing other means of fixation and probably lyophilizing techniques. However, since the particles which have been observed had complex shapes, they cannot be dismissed lightly. Furthermore, these shapes are related to what the light microscopist can observe in living material as well as in fixed preparations. Finally, we have presented incontrovertible evidence that the particles are not greatly hydrated, so that there is no reason to expect major changes. The principal artifact may well amount to nothing more than some shrinkage.

We have emphasized that the hydrated matrix of the chromosome was not well preserved; we believe, nevertheless, that we often have seen chromonemata. In electron micrographs with good resolution thin fibrils commonly were seen extending away from unit particles toward adjacent bands. Usually they were attached to the tapered tips of particles, but the globular particles sometimes seemed to be attached at one side. A few micrographs showed these filaments in great profusion. Under favorable circumstances, a single fibril could be traced from a particular particle of one band to one of the next as in Fig. 4B. Where these fibrils are most conspicuous in the figures, they have been designated by the letter N. No clearly defined chromonemata have been observed that were thicker than about 80 A. The thinner fibrils, probably stretched, were sometimes less than 50 A thick.

Even though this work must be regarded as in its early stages, we have been impressed by the close

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colloids, quantum theory, photochemistry, and nucleonics are included. Emphasis is placed on the structure of matter, and not until this fundamental groundwork has been treated is the student exposed to the more abstract thermodynamic development of the subject. Practically all the necessary mathematical derivations are given in detail in the text; only a few are relegated to an appendix for the ambitious student. The annoying tendency of many writers to overwork the phrases "it can be shown" and "obviously" does not appear.

One of the best features of this text, from the pedagogical point of view, is the liberal use of numerical examples and problems. Furthermore, these are not hypothetical cases which fail to give the student any contact with reality, but are mainly based on actual data from the literature. It is the sort of training which will best prepare the student for research work later. These problems cover the whole range of practical work from calculation of atomic weights from gas densities to determination of the rate of transmutation in an atomic pile. Another commendable feature of the book is the bibliography: references to up-to-date literature are given, and, in addition, each chapter concludes with a series of cross-references to other books for supplementary reading. It is a pleasure to recommend *Outlines of physical chemistry*, and Prof. Daniels is to be congratulated for a fine piece of work.

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North American trees (exclusive of Mexico and tropical United States). Richard J. Preston, Jr. Ames, Ia.: Iowa State College Press, 1948. Pp. lv + 371. (Illustrated.) $4.00.

Setting as his triple target the nontechnical public, students, and scientists, the author should be credited with a near miss. For the nontechnical public and for beginning students of dendrology, this compilation of illustrations and almost telegraphic descriptions of the important trees of the United States and Canada should prove very helpful. Certainly, it will save the time of those who, in the past, have had to consult five or six important works for various geographic regions. But the scientist will wish to digest for himself the authoritative source material which the author has assimilated but failed to cite in any list of references in this volume.

Eleven pages, packed full of technical terminology and diagrams, are devoted to a *nontechnical* explanation of the "Natural Relationship of Trees" and to descriptions of the "Forest Regions of North America" and "Tree Characters." Had the frontispiece map been slightly modified to coincide with the groupings of the forest regions in the text, this subject of regions and forest types would have been even more useful.

The Keys to the Genera—the keys to the species of the genera among the gymnosperms and to those of the genera among the angiosperms—will, with the liberal use of the 9-page glossary, prove very useful indeed. However, the chief value of the book would seem to lie in the compact presentation of drawings of foliage, twig, bud, fruit, and seed characteristics in a form convenient to take into the field for direct comparison. The small distribution maps are also helpful.

The volume is recommended to those who are frequently plagued with the question: "What's that tree?"

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Scientific Book Register

COURANT, R., and FRIEDRICHS, K. O. *Supersonic flow and shock waves.* New York-London: Interscience, 1948. Pp. xvi + 464. (Illustrated.) $7.00.

HARRISON, GEORGE R., LORD, RICHARD C., and LOOFBOUROW, JOHN R. *Practical spectroscopy.* New York: Prentice-Hall, 1948. Pp. xiv + 605. (Illustrated.) $6.65.

MAYER, CLAUDIUS F. (Ed.) *Index-catalogue of the library of the Surgeon General's office, United States Army (Army Medical Library).* (Fourth Series, Vol. X, M-Mez.) Washington, D. C.: Superintendent of Documents, U. S. Govt. Printing Office, 1948. Pp. iv + 994. $4.25 (cloth).

POLLARD, ERNEST C., and STURTEVANT, JULIAN M. *Microwaves and radar electronics.* New York: John Wiley; London: Chapman & Hall, 1948. Pp. vii + 426. (Illustrated.) $5.00.

SCHMIDT, ALOIS X., and MARLIES, CHARLES A. *Principles of high-polymer theory and practice.* New York-London: McGraw-Hill, 1948. Pp. xii + 743. (Illustrated.) $7.50.

SEWELL, R. B. SEYMOUR. *The free-swimming planktonic Copepoda: geographical distribution.* (The John Murray Expedition, 1933–34; Sci. Rep., Vol. VIII, No. 3.) London: British Museum (Natural History), 1948. Pp. 318–592. (Illustrated.) 35/–.

SMITH, AUSTIN, and HERRICK, ARTHUR D. (Eds.) *Drug research and development.* New York: Revere, 1948. Pp. xi + 596. $10.00.

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agreement of our findings with the predicted theory of the ultrastructure of the chromosome. The unit particles observed by us were associated with individual chromonemata. Although the particles varied in shape and size from one band to another, they had a uniform character in any one band. Their size range and density make them comparable with virus and bacteriophage particles. They are identifiable with the substance of chromomeres well known to the light microscopist. There is no doubt but that this is nucleoprotein. In view of these conclusions, it seems reasonable to believe that the discrete particles we have seen are genes.