Smear Preparations for the Electron Microscopy of Animal Chromosomes[1]

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AN ESSENTIAL REQUIREMENT for an electron microscope preparation is that it pass the test of light microscopy: its structure should appear at least as well preserved as the best conventional preparations for cytological study. The most useful procedure for the analysis of chromosome structure in the light microscope has been the so-called squash or smear technique. The squashing or smearing of cells between slide and cover slip is used to break the nucleus, thus setting the chromosomes free for study, and furnishing specimens with regions that are stretched so thin that details of the internal structure can be looked for. Until the present work, no successful routine had been developed by which the smear technique could be adapted to furnish electron microscope preparations.

Methods previously employed for the electron microscope study of chromosomes include isolation of the individual chromosomes by microdissection (*1, 3–5*); the preparation and isolation of chromatin threads by differential centrifugation of disrupted nuclei (*6*); and the cutting of very thin sections of embedded tissue (*4, 8*).

The procedure to be described here is an adaptation of the standard cytological smear technique, and provides preparations suitable for study in both light and electron microscopes.

1. *Primary fixation and squash.* The first step in the procedure is carried out as for conventional light microscopy. In making good squash preparations one must fix the material rapidly enough to preserve structure, without hardening it so much that it cannot be stretched. A fixative-stain containing 2 percent orcein and 60 percent acetic acid is satisfactory for both types of cell studied here, *Drosophila* salivary gland cells and the spermatocytes of man. The material can be dissected either in the appropriate Ringer's solution, or in the fixative-stain. In either case, dissection should be followed by about ten minutes in the fixative-stain, to a fresh drop of which the material is then transferred. A clean cover slip is mounted over it, held at one side with a piece of blotting paper, and tapped with a needle under the dissecting microscope, first to spread the cells into a single layer, then more forcibly to break the nuclei. They are then compressed still further by rolling the thumb, with maximum force, over a fresh piece of blotting paper placed upon the cover slip to remove excess fluid from the preparation.

2. *Transfer to formvar film.* The preparation is frozen over a block of dry ice, after which the cover slip can be pried off with a chilled razor blade. Part of the material then adheres to the slide and part to the cover slip. On the desired part of the preparation is then placed a drop of 95 percent glycerine and 5 percent saturated aqueous solution of lanthanum acetate. The lanthanum acts as an additional fixative, important for preserving the structure through the later treatments, and possibly serves as an electron stain for the nucleic acids.

A slide or cover slip previously coated with a formvar film approximately 200 A thick is quickly placed over the cellular material. Inserted into the jaws of a vise, the preparation is pressed firmly against the film, but not so forcibly as to smash the chromosomes. The preparations are now examined under the light microscope, and failures are discarded.

Slide and cover slip together are now placed in a Coplin jar containing a third fixative, a solution of three parts saturated picric acid, and one part formalin (40 percent formaldehyde). After six to twelve hours in this solution, the slide and cover slip will have separated, leaving some of the chromosomes attached to the formvar film.

3. *Dehydration.* The preparation on the film is now rinsed in distilled water and carried through the usual series of increasing concentrations of alcohol,

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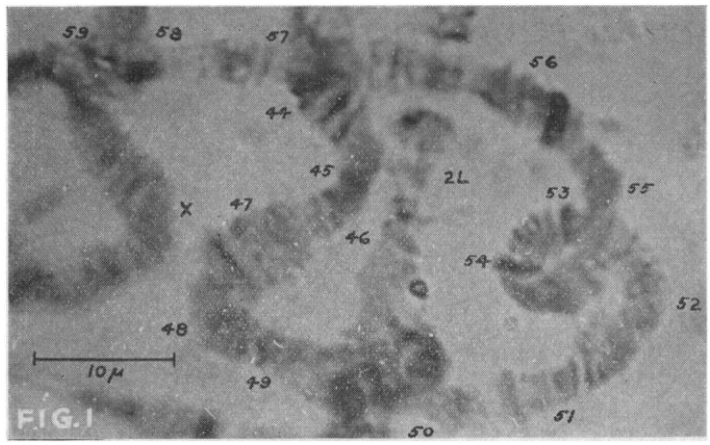

FIG. 1

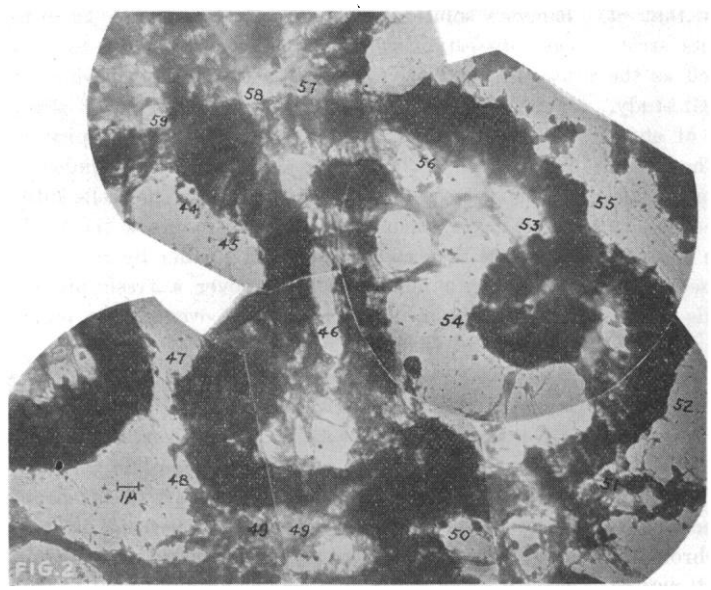

FIG. 2

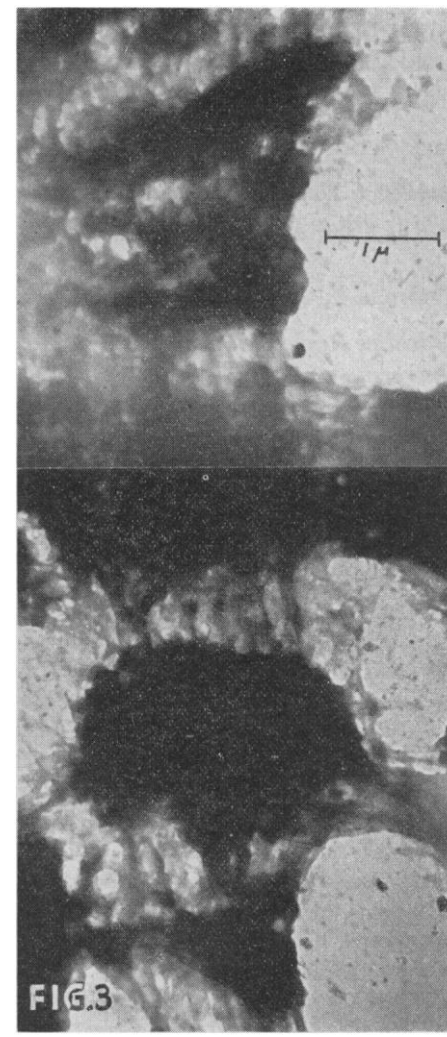

FIG. 3

and finally through two changes of absolute alcohol. Following this, it is placed in a 0.5 percent solution of collodion in absolute alcohol for ten minutes. On removal, the preparation is allowed to dry in air and, being imbedded in collodion, suffers little or no distortion.

Extractable collodion is then removed with absolute alcohol, two changes of two hours each. The preparation can now be dried once more, without visible damage to the structure.

4. *Selection of region for study.* The preparation is ready for an exploratory examination under the high dry objective. The region found appropriate for study is cut around with a dissecting needle and teased up to permit a drop of water to flow under the plastic film (*9*), the drop being confined by a ring drawn with a wax pencil. The supporting grid for use in the electron microscope is then slipped under the film and the water is drawn off with a piece of blotting paper. A small amount of detergent in the water facilitates wetting the grid.

5. *Examination under oil immersion (light microscope).* The dried preparation, screen and all, may now be immersed in oil—cedar oil, for example—so that favorable regions can be studied and photographed under optimal conditions for light microscopy. By gentle washing in chloroform, the oil may be removed from the specimen, which is then ready for study in the electron microscope.

An example of the results obtained by our procedure is shown in Fig. 1. It is a light microscope photograph of part of a larval salivary gland nucleus of *Drosophila melanogaster*. Larvae from homozygous Oregon R stock were used, containing mutants *gt* and w^a. We have identified the chromosome regions according to the maps of Bridges (*2*). Sections 44 to 59 of chromosome 2R are outlined by the rectangle of the figure. Unidentified portions of chro-

mosomes X and 2L can also be seen. In Fig. 2, a montage of a series of electron micrographs of this same section is shown. It is distorted somewhat by breaking of the formvar film. It is clear that the

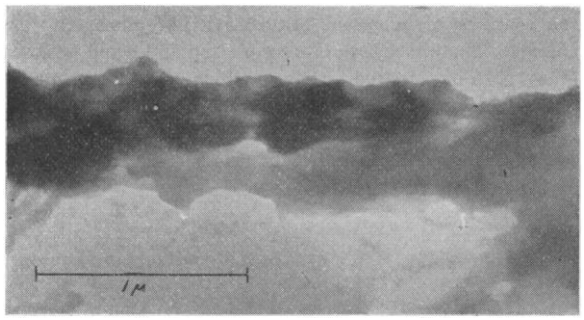

FIG. 4.

bands of the light microscope map, corresponding to the Feulgen-positive, ultraviolet-absorbing regions shown to be nucleoprotein in character, are also the regions of greatest density to the electron beam.

In Fig. 3, a higher magnification shows fine structures. The bands are seen to be composed of a multitude of particles like those seen in the thin sections of Pease and Baker (*8*), and in the replica preparations of Palay and Claude (*7*) demonstrated in a lecture at the University of Pennsylvania. Pease and Baker have made a somewhat detailed discussion of the fine structure of their preparations. Our results, so far as they go, are not in contradiction to theirs. However, it is too early to give support to their suggestion that the character of a band is determined by the size and shape of the individual particulate bodies of which it is composed.

The less dense interband regions show varying numbers of fibrils extending from one band to the next. On these fibrils small globules can be seen, sometimes regularly, sometimes irregularly spaced on adjacent fibrils. The irregularities are perhaps due to unequal stretching. If the globular particles represent types of bands, they would be below the limits of resolution of the light microscope. The fibrils that carry the globules have roughly the same diameter within one interband space. The larger fibrils may, however, be aggregates of smaller ones, some of which are just resolved. It would be premature to discuss the relation of these fibrils to theories of the structure of the giant chromosomes.

Fig. 4 shows an electron micrograph of a similarly prepared human pachytene chromosome, from the same type of material as that used in the study of the nucleolar chromosome of man by Schultz and St. Lawrence (*10*). Here also the alteration of density, corresponding to chromomere and interchromomeric regions, can be seen. The picture corresponds quite well to the studies in similar stages in *Lilium* (Elvers) and *Zea* (Buchholz). In the present micrographs it is possible to see a fine structure of fibrils in the interchromomeric regions (interbands).

The identification of genes with definite areas of the chromosomes of *Drosophila* was made possible as the result of a correlated study of the genetics and cytology of chromosome rearrangements. With the present technique, it is evident that the study can be extended to the electron microscope, and a basis laid for a discussion of the relation of the ultrastructure of the chromosomes to the genes. Similarly, as in human material, where chromomeres are at the limit of resolution of the light microscope, the use of the electron microscope is essential to further progress in mapping chromosomes.

References

1. BOCHE, R. D. and ANDERSON, T. F. Cited in article by G. A. Morton, *RCA Rev.*, 1941, **6**, 131.
2. BRIDGES, C. B. *J. Hered.*, 1935, **26**, 60.
3. BUCHHOLZ, J. B. *Science*, 1947, **105**, 607.
4. ELVERS, I. *Acta Horti Bergiani*, 1943, **13**, 150.
5. FRIEDRICH-FREKSA, H. *Naturwiss.*, 1940, **28**, 376.
6. HOVANITZ, W. *Genetics*, 1947, **32**, 500.
7. PALAY, S. L. and CLAUDE, A. *J. exp. Med.*, 1949, **89**, 431.
8. PEASE, D. B. and BAKER, R. F. *Science*, 1949, **109**, 8.
9. PORTER, K., CLAUDE, A., and FULLAM, E. F. *J. exp. Med.*, 1945, **81**, 233.
10. SCHULTZ, J. and ST. LAWRENCE, P. *J. Hered.*, 1949, in press.