Technical Papers

Electron Microscopy of Chromosomes in Smears[1]

Israel J. Heilweil, Harriet G. Heilweil, and Quentin Van Winkle[2]

Department of Chemistry, The Ohio State University, Columbus

In a previous communication from this laboratory, Van Winkle *et al.* (*1*) reported the results of electron microscopical studies on isolated resting chromosomes. Concurrently, techniques were developed for the electron microscope investigation of the giant salivary gland chromosomes of *Drosophila melanogaster* and the meiotic chromosomes of the microsporocytes of *Rhoeo discolor* in smear preparations.

Electron microscope studies of plant chromosomes isolated by a microdissection method have been reported by Buchholz (*2*). Drosophila giant chromosomes have been examined electron microscopically in smears by a negative replica method (*3*) and by the smear transfer method of Schultz *et al.* (*4*).

In order to investigate chromosomes in smears that have been chemically treated so as to reveal details of chromosomal structure and composition, a simple and easily mastered technique for transferring entire smears from glass slides to electron microscope specimen-supporting screens was adapted from a procedure described by Manton and Smiles (*5*). Drosophila salivary glands were dissected out in 45% acetic acid, fixed in this reagent for 10 min, and smeared in the usual manner. Anthers of Rhoeo were first smeared on carefully cleaned slides with a flat-honed knife, fixed in 45% acetic acid for 10 min, and then flattened. Cover glasses were removed according to a method described by Schultz *et al.* (*4*), which involves freezing the slides on dry ice for about 10 min and prying up the cover glasses with a chilled razor blade. Subsequently, the slides bearing the smears were placed in a slide carrier and passed through a given series of reagents.

If the smears were to be transferred to electron microscope screens immediately after fixation, the slides were passed successively, for 15-min periods, into solutions of glacial acetic acid and absolute ethanol in the proportions 1:1, 1:3, 1:9, and 0:1. They were then washed in amyl acetate and dried in a vacuum desiccator. Each individual slide was next dipped vertically into a 1:4 solution of collodion and amyl acetate, removed slowly and steadily, drained of excess solution by touching the end of the slide to absorbent paper, quickly placed in a vacuum desiccator in a vertical position, and dried for 30 min at room temperature. The following day, small rectangles of film were stripped from the slide by an adaptation of a method devised by Martin and Tomlin (*6*). After a suitable area of the smear was located by means of the phase microscope, three sides of a rectangle approximately 3×5 mm, and surrounding the selected area, were cut with a sharp, single-edged blade. The slide was then held in a dish of water at a 45° angle so that the rectangle was completely immersed, its uncut narrow edge uppermost. Under a dissecting microscope the lower cut edge of the rectangle was teased up, and the flap was pulled gently with carefully ground square-ended forceps. When the rectangle was free of the slide except at its uncut edge, the slide was removed from the water in such a way that the rectangle returned to its original position. The fourth side was then cut through, and the slide immersed vertically into a dish of water so that the rectangle of film floated off onto the surface of the water. The freed rectangle was picked up on an electron microscope specimen screen by bringing the screen up under it. After touching the edge of the screen to filter paper, the specimen was dried over phosphorus pentoxide overnight.

Some smears, fixed in acetic acid, with cover glasses removed, were subjected to a procedure devised by Bretschneider (*7*) and described by Van Winkle *et al.* (*1*), which is believed to localize desoxyribonucleic acid in electron micrographs. They were subsequently dehydrated by carrying the slides through water-ethanol solutions of increasing ethanol concentration. They were then embedded in collodion film, stripped, and mounted on electron microscope screens as outlined above. In other experiments, acetic acid–fixed smears were dehydrated in the glacial acetic acid–absolute ethanol sequence followed by two additional changes of absolute ethanol, dried in air, and digested for 1 hr in a solution of desoxyribonuclease at 40° C according to the procedure of Palay and Claude (*3*). The enzyme solution was prepared by dissolving 0.01 mg of the enzyme per ml of phosphate buffer of pH 7.3 made 0.005 *M* in $MgSO_4$. Following digestion, the slides were washed in five changes of water and three changes of absolute ethanol. They were dipped, finally, in the collodion film-forming solution, and the film was stripped and mounted as before.

All electron micrographs were made by means of an RCA Model EMU instrument.

The condensed stages of the meiotic chromosomes of Rhoeo were generally opaque even after conventional pretreatments, hydrolysis in 1.0 *N* HCl for 6 min, or the Bretschneider procedure. Structural details that would be beyond the limits of resolution of light microscopes, however, were observed in well-smeared chromosomes in late second telophase and

[1] These studies were aided by grants from the National Institutes of Health of the Public Health Service, E. I. du Pont de Nemours & Company, Abbott Laboratories, and the Chemistry Department of The Ohio State University.

[2] The authors wish to express their gratitude to Albert F. Prebus, Elton F. Paddock, and Allen S. Fox for their valuable assistance.

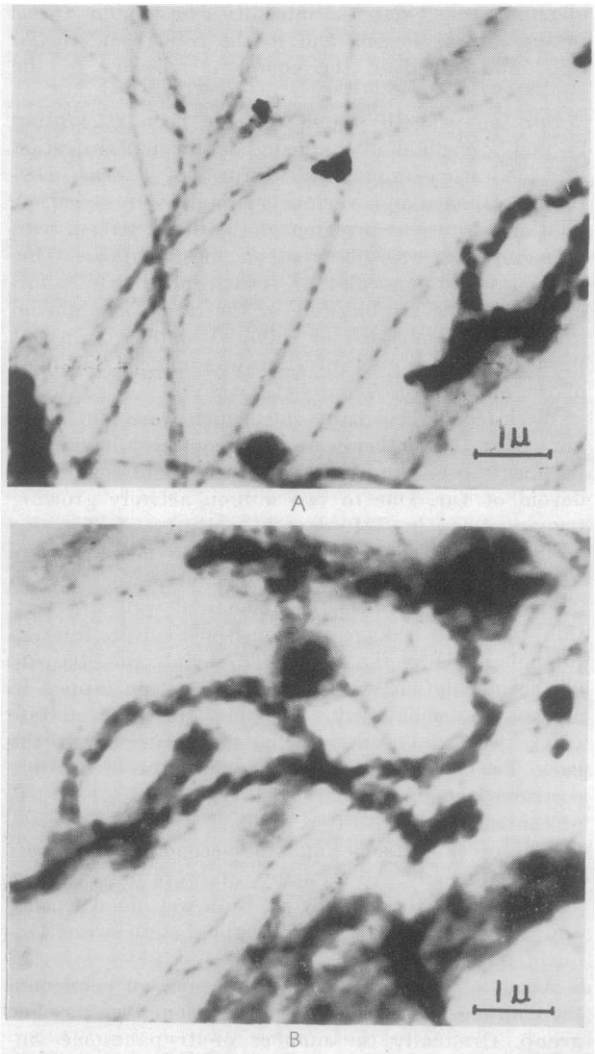

FIG. 1. Smear of microsporocyte of *Rhoeo discolor* in late second telophase fixed in 45% acetic acid.

early prophase stages. Electron micrographs of late second telophase chromosomes fixed only in acetic acid (Fig. 1) indicate that these chromosomes are made up of undetermined numbers of tubules, about 0.1 μ in diameter, twisted about each other, often in pairs, and having distributed within them dark segments approximately 100 × 50 mμ in cross-sectional area. It appears that the twisted tubules are enclosed in a casing. The apparent spiraled structure of chromosomes, as at the lower right of Fig. 1 *A*, may be due to the arrangement of the dark segments of the twisted tubules. Identical structures were seen in electron micrographs of late second telophase chromosomes treated according to the Bretschneider procedure. The diameters of the tubules agree in order of magnitude with those of the tubules observed by Van Winkle *et al.* (*1*) in suspensions of isolated animal chromosomes which had undergone the Bretschneider treatment. In their general appearance, the tubules closely resemble the chromatin strands isolated by Claude and photographed in the light microscope (*8*).

When salivary gland smears of Drosophila were fixed only in acetic acid and then transferred by the procedure described above and examined in the electron microscope, bands ordinarily observed in the light microscope were seen to be composed of several narrower bands. In addition, numerous fine bands were discerned in the so-called interband regions. Fig. 2

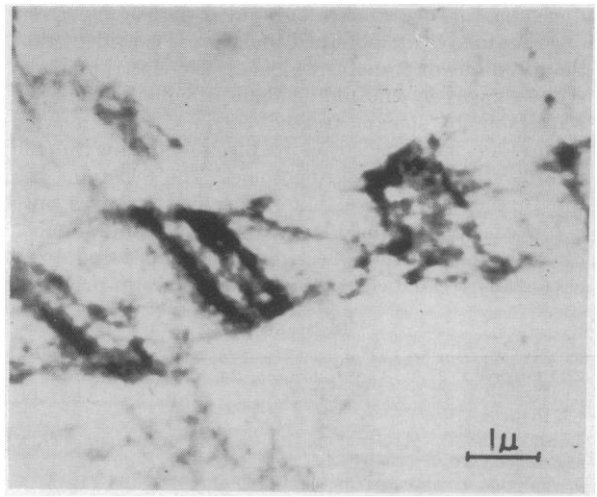

FIG. 2. Salivary gland chromosome of *Drosophila melanogaster* treated according to the Bretschneider procedure.

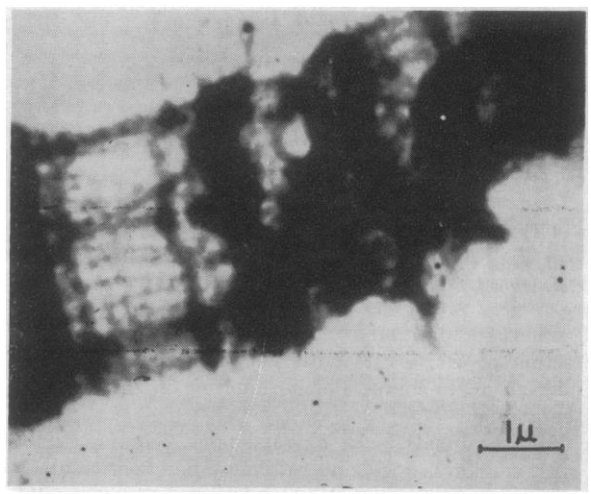

FIG. 3. Drosophila salivary gland chromosome treated with desoxyribonuclease.

shows a portion of a salivary gland chromosome subjected to the Bretschneider procedure. Apparently this treatment removes a considerable amount of material from both the band and interband regions and reveals the finest bands to be transverse rows of granules roughly 30 mμ in diameter. Moreover, fibers appear to connect the rows of dark granules in an irregular fashion. Similar details are seen in the desoxyribonuclease-digested chromosome of Fig. 3, but the trans-

verse rows of granules and the generally longitudinal connecting fibers are more regularly arranged. Smears of silver-stained (*1, 7*) salivary gland chromosomes were also examined in a microscope using visible light. It was found that the band regions were strongly stained by silver, whereas the interband regions were either slightly stained or not stained at all. The total lengths of the chromosome arms were not appreciably altered by the silver-staining procedure, but the widths of the arms were considerably reduced. The nucleolus was observed to be stained slightly by silver.

From the results obtained thus far, it is evident that the technique of transferring smears to electron microscope screens by embedding them in collodion films is applicable to smears treated chemically by a wide variety of methods so as to disclose details of the internal structure of chromosomes. The technique has the further advantage that in itself it involves only a few easily performed steps and a minimum number of reagents. Further electron microscope studies of chemically treated chromosome smears are in progress.

References

1. VAN WINKLE, Q., *et al. Science,* **115**, 711 (1952).
2. BUCHHOLZ, J. T. *Am. J. Botany,* **34**, 445 (1947).
3. PALAY, S. L., and CLAUDE, A. *J. Exptl. Med.,* **89**, 431 (1949).
4. SCHULTZ, J., MACDUFFEE, R. C., and ANDERSON, T. F. *Science,* **110**, 5 (1949).
5. MANTON, I., and SMILES, J. *Ann. Botany,* **7**, 195 (1943).
6. MARTIN, A., and TOMLIN, J. O. *Biochim. et Biophys. Acta,* **5**, 154 (1950).
7. BRETSCHNEIDER, L. H. *Proc. Koninkl. Nederland. Akad. Wetenschap.,* **52**, 301 (1949).
8. CLAUDE, A. *Harvey Lectures,* **43**, 121 (1948).

Manuscript received January 11, 1952.

Fatal *Trypanosoma cruzi* Infection in the White Rat[1]

Harry Seneca and Joyce Rockenbach

College of Physicians and Surgeons, Columbia University, New York

This is a preliminary report on the laboratory infection of white rats with *Trypanosoma cruzi,* running an acute course and terminating in the death of the animal.

Many animals, such as rats, mice, lemurs, guinea pigs, monkeys, dogs, and cats, may be experimentally infected with *T. cruzi.* Mice and guinea pigs are especially useful in diagnostic and research work on Chagas' disease (*1*). Inoculations of white rats with citrated blood from the patients have been successful in some cases. The guinea pig is less useful because it is less susceptible to infection than the white rat (*2*). Baby rats inoculated intraperitoneally with large doses of *T. cruzi* cultures show a few trypanosomes in the blood after an incubation period of 10 days to 2 weeks. This mild transient parasitemia, lasting about 2 weeks, was associated with leishmania forms in the cardiac muscle and in the reticuloendothelial cells, and ended in the complete recovery of the animal (*3*).

For the past 10 years, we have been attempting to find a method of inducing acute fatal infection in a laboratory animal with cultures of *T. cruzi.* Different animals and various methods were tried. A mild and transient invasion of the blood stream with trypanosomes was observed in most animals. One method was to inoculate *T. cruzi* cultures into animals following the blocking of the reticuloendothelial system with India ink or colloidal metals. Transient parasitemia with mild infection was induced, but most of the animals recovered (*4*).

Week-old, hybrid baby rats which were still being nursed by the mother were used routinely in our experiments. The skin was thin, tender, and completely devoid of fur. One to two million actively growing trypanosomes in 7–10-day-old cultures of *T. cruzi* from bi-phasic Seneca hemoflagellate medium were injected intraperitoneally in 0.2 ml saline with a 24-gauge needle. Since the abdominal wall was very thin, there was leakage of the injected culture through the hole made by the needle. To obviate this, the site of the inoculation was rubbed with cotton dipped in alcohol. Concomitantly, 6.25 mg cortisone acetate (Merck, 0.25 ml) was injected subcutaneously in the back. Twenty-four hr later, a second dose of 6.25 mg cortisone acetate was injected. There were two sets of controls for each litter. One set was injected with two doses of cortisone, and the second with *T. cruzi* intraperitoneally. All the baby rats were put together, and nursed by the mother rat. Both wet blood smears, and smears stained with Wright's stain were frequently examined.

About a week after the inoculation, an occasional trypanosome was seen in the cortisone-trypanosome group. Gradually the number of trypanosomes increased, so that in 3–4 weeks, there were 25–100 trypanosomes per high-power field of the microscope. Some of the animals died of severe parasitemia in 3 weeks after inoculation. Inhibition or retardation of growth, loss of appetite, loss of weight, tendency to sluggishness, rough texture of the fur, eye infections, and gradually increasing paralysis of the hind legs were observed. Within 5 weeks all the inoculated animals died of muscular paralysis, which gradually spread to the muscles of respiration. Rats inoculated with trypanosomes only (controls) showed a mild transient parasitemia about 2 weeks after injection; the parasitemia lasted 10 days to 2 weeks, and terminated in complete recovery. Cortisone controls showed inhibition and retardation of growth, poor texture of fur, and reduced resistance to bacterial infection, but continued to live.

Post-mortem examination of the cortisone-trypanosome group showed congestion of the meninges, brain, and liver. The spleen was enlarged, and the heart dilated. No gross pathological changes were observed in the other organs. Sections of the tissues showed

[1] This study was supported by a grant from Chas. Pfizer & Co., Brooklyn, N. Y.