# ULTRASTRUCTURE ANALYSIS OF A PORTION OF THE X-CHROMOSOME OF *DROSOPHILA MELANOGASTER*

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by

Albert S. C. Lossinsky

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H. Michael LeFevers
Approved for Major Department

Howard E. Davis
Approved for Graduate Council

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Sincere thanks is also extended to those members of my committee including Dr. Helen McElree and Dr. Katherine Smalley, as well as Mr. Russel C. Moore, my friend and collegue who was of great assistance in the development of the tissue squash preparations.

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# INTRODUCTION

A single DNA molecule is an exquisitely thin filament, so fine that a length sufficient to reach from the earth to the sun would weigh less than one-half a gram. The human body is said to contain between 10 and 20 billion miles of DNA double helix distributed among some trillion cells. Clearly, there must be some very well developed mechanisms for the packaging of the DNA into chromosomes, as well as extraordinarily intricate mechanisms for the uncoiling of specific, selected moieties of the molecules for protein synthesis, transcription, replication and chromosome repair (DuPraw 1970).

Current research investigations in a variety of disciplines related to the field of genetics, including molecular genetics, biochemical genetics, cytogenetics and others, have yielded significant advances to the overall conception of chromosome structure and function. Such advances have been made possible by the development of an assortment of highly sophisticated tools for such research including high resolution electron optics, application of radioisotopes, and cytochemical methods. These advances are now elucidating discrepancies that have existed for many years. Such methods, in correlation with various analytical techniques, are directing science to the inner sanctums of chromosome-gene interaction.

One item of current investigation that has contributed significantly to the understanding of the chromosomes is the study of the large salivary gland chromosomes of the Dipterans, namely members of the genus Drosophila. Within the nuclei of the salivary glands exist rather unique polytene

chromosomes (Berendes and Beerman 1969). The uniqueness of these chromosomes lies in their extraordinary size, ie, some 100-150 times the size of germ cell or somatic chromosomes (Swanson, Merz and Young 1967).

In addition to the salivary gland, the giant chromosomes are generally found in the cells of other well differentiated organs involved in vigorous metabolism such as those of the intestine, rectum, Malphigian tubules and a varity of other cell types in both plants and animals. Other genera in which polytene chromosomes have been identified include Sciara, Chironomus, Rhynchosciara and Eusimulium (DuPraw 1970).

Apparently, tissue growth in these cell types is unlike normal mitotic conditions as there is an increase in cell size rather than in cell number (Beerman and Clever 1964). Ross (1939) observed the development of the salivary gland cells of Drosophila melanogaster. He demonstrated that in the initial stages of their development, these cells undergo mitosis; however, after 18 hours of development, this cellular proliferation ceases and is followed by the cellular distension described above. These particular cells then, are adapted for interphase synthesis.

The increased dimensions of the polytene chromosomes, reaching up to 2mm in length and up to 5μ in diameter are attributed to the fact that each chromosome begins as a single strand of DNA, then enters into a replication phase (Swanson, Merz and Young 1967). This duplication continues a number of times resulting in a juxtaposition of 1000-4000 homologous chromatid strands (DuPraw 1970). DuPraw and Rae (1966) define these strands as unit chromatids. Each chromatid is thought to contain a single DNA molecule (DuPraw 1970).

These polytene chromosomes contain an intricate organization of genetic material in association with a number of additional matrical substances. The polytenes have been compared to the meiotic chromosomes in the pachyneme stage, ie, a type of somatic pairing (Swanson, Merz and Young 1967). This is also thought to be where the term polyteny originated.

Examinations of salivary gland squash preparations under phase optics demonstrate the existence of cross striations or bands throughout the length of the chromosome. These bands are thought to result from a coiling of the densely packed, polypartite, nucleoprotein material in these specific regions (Beerman and Clever 1964). The areas between the coiled areas are termed the interbands and contain less densely packaged DNA compared to the band regions (Sorsa and Sorsa 1967b).

The polytene chromosomes have been compared to a number of structures including an electrical cable (DuPraw 1970), as well as to the structure of a rope (Beerman and Clever 1964). The later concept can be used to illustrate a chromosome model by simply visualizing a rope with knots periodically tied along its length. The rope is constructed by a number of parallel strands that intertwine each other. These knots then, exist in a supercoiled state, and can be easily visualized as the chromosome bands and the untied areas as the interbands. Figure 1 depicts this type of coiling and exemplifies the puffing regions that occur along certain positions on the chromosome.

Although a complete discussion concerning the biochemical composition of these polytenes will not be emphasized in this paper, it is, however, necessary to offer brief biochemical comments in order to fully appreciate

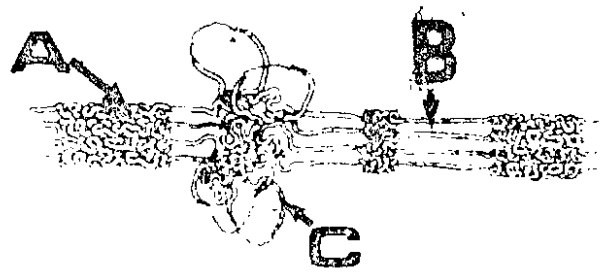


Figure 1. — This figure exemplifies the dense coiling of the band regions (A) and the less dense interband regions (B). A puff is shown (C) as the nucleoprotein uncoils in specific regions of the chromosome for RNA synthesis (DuPraw and Rae 1966).

these entities at their fine structural levels. Considerable conclusive evidence supports a generally conceived idea that the polytene chromosomes of Drosophila melanogaster consist of a number of DNA fibers in association with a great deal of protein. These proteins include the major histone classes, nonhistone proteins such as RNA polymerase and the enzyme for histone acetylation (DuPraw 1970). In Drosophila salivary gland chromosomes, Boyd, Berendes and Boyd (1968) have measured the chemical compositions of these chromosomes. They reported that the composition is approximately 24.8% DNA, 28.5% acid soluble protein (histone), 38.4% acid insoluble protein and 8.4% RNA.

The histone content has been shown to exhibit the same marked proportionality to DNA that has been observed in less complex interphase nuclei (Swift 1964) and appears to serve important functions within the chromosome. One theory is that the histones serve as a backbone supporting the interweaving DNA molecules. Mazia and Jaeger (1939) suggested that this protein group closely followed the DNA distribution within the chromosomes.

Soon after cytologists developed DNA specific stains, it was established that the chromomeres (bands) of the polytenes were the regions of high DNA concentration compared to lower concentrations in the interbands. It remained a controversial issue for many years as to whether the interbands contained any DNA at all. It later became evident with the development of the Feulgen Stain, fluorochromes, and radioactive labeling techniques that the interband indeed contained low concentrations of the nucleic acid.

Following investigators then began to entertain the possibilities of identifying specific regions along the chromosomes where DNA was located. These efforts resulted in the overall assumption of a DNA continuity along the length of the chromosome. This DNA concentration appeared to be more concentrated in the banded areas compared to the interband regions. DuPraw and Rae (1966) estimated this chromomeric DNA concentration. It was found that the average DNA packing ratio from the total length of the DNA double helix in a Drosophila haploid chromosome set (ca. 16cm), divided by the entire length of the chromosome after stretching (2219μ) suggested an average packing ratio of 70:1. It is evident that there must be remarkably efficient mechanisms not only for the packaging of this nucleoprotein material in the bands, but also for its unwinding the double helix locally to repair accidental breaks (DuPraw 1970).

The polytenes also serve as an excellent tool for investigating RNA synthesis within these chromosomes. Using RNA specific stains or radioactive labels, it is possible to locate these areas. Such regions are thought to be the areas of high metabolic activity identified by swollen sections along the chromosome, ie, the puffs or Balbiani rings (Figure 1). The nucleoli of these salivary gland cells are specialized RNA-containing structures. These nuclear organelles, when present, often appear attached to the chromosome at specific positions, the nucleolar organizing regions (Berendes and Beerman 1969).

Historically, perhaps the earliest observation of the polytene chromosomes was made by Balbiani (1881) who can and should be visualized

as the "Father of Polytene Chromosomes" since he clearly identified large banded structures in Chironomus larval salivary glands. Alverez (1912) suggested that the band numbers differed with respect to the age of the Chironomus larvae. Kostoff (1930) also made observations of the large chromosomes of the salivary glands of Drosophila melanogaster. Although he failed to support his experimental data, he did describe disc structures which were reported to have high affinity for haematoxylin stain, suggesting that he was observing structures that housed "packages of controlled inheritable characteristics."

The 1930's proved to be an era filled with many investigations of chromosome structure setting springboards for many later research efforts in chromosome structure and function. Heitz and Bauer (1933) reported banding patterns found in polytene chromosomes in Malphigian tubules of certain fly genera. Painter (1934b) and a number of others began this era by offering a number of cytological maps of the polytenes and outlined early relationships between gene location and function. Koltzoff (1934) followed to further elucidate polytene structure by suggesting that the "axial threads" of the chromosomes were the specific sites of gene location. These "genenemes," as they were termed, are the positions on the chromosomes that are the sites where the interbands are located.

Muller and Prokofyeva (1935) analysed results of irradiated X-chromosomes of Drosophila melanogaster. They proposed the hypothesis that a one to one relationship existed between band and gene numbers, ie, the one band-one gene hypothesis. They prepared cytological maps with specific gene positions on the chromosomes corresponding to specific "nodes" as

they were termed.

Further band number investigations of the salivary gland chromosomes were undertaken by Bridges (1935) who proposed, from light microscopic observations of squash preparations, that the X-chromosome of Drosophila melanogaster contained some 725 bands across its entire length. A revision of this map using similar methods indicated an increase in the band count of the X-chromosome by 41%, ie, an increase from 725 to 1024 bands (Bridges 1938). It was also observed in this later study that the length of the chromosome similarly increased, ie, from 220μ (Bridges 1935) to 414μ. The rationale for this marked increase was that the smaller, less stretched chromosome failed to show all of the "faint" bands, and perhaps more importantly, many double bands (doublets) in the unstretched chromosomes were observed and recorded as single bands (singlets).

The analogy of the polytene chromosome to an accordian is useful in understanding the morphology of the doublet. "Unless these chromosomes are stretched, the doubleness of most bands is not visible and many fine or dotted lines are obscured by their appressed neighbors" (Bridges 1935). Thus it appears that the number of doublets observed by various investigators differed depending upon the quality of chromosome squash preparation obtained.

The reality of the existence of the doublet character of many bands in the revised map of Bridges (1938) has been questioned (Beerman 1962; Berendes 1968). This is due to the calculation made by Bridges (1942) of 1299 double bands from a total of 5161 bands among all of the arms of the salivary gland chromosomes of Drosophila melanogaster (Cooper 1959).

The use of the transmission electron microscope, with higher magnification and resolution capabilities than that of the light microscope has been a tremendous tool for studying the polytene chromosomes. Although the electron microscope was in its infancy in the late 1940's and early 1950's, a number of polytene chromosomes were investigated during this era (Heilweil, Heilweil and van Winkle 1952; Herskowitz 1952; Borysko 1953; Beerman and Bahr 1954; Gay 1955; Loman 1956; Kaufman and McDonald 1956 and others). The quality of resolution was inferior since proper squashing techniques were not yet developed and mapping of chromosomes from whole cell preparations was impractical.

More recently, Berendes (1968; 1970) employed the transmission electron microscope in an investigation of the ultrastructure of the tip of the X-chromosome of Drosophila melanogaster in attempts to further understand the controversial doublet phenomenon. He suggested that the presence of observable doublets may have significant bearing on the correlation between cytological and genetic fine structure as well as in calculating the average size of a band in terms of haploid DNA content as suggested by Berendes and Beerman (1969). The average DNA content of a single band was calculated to be $0.9 \times 10^{-4}$ picograms.

Investigating the X-chromosome, Berendes (1970) compared electron microscopic observations to the revised cytological map of Bridges (1938) in map Regions 1A-4E. Based on observations of 31 different chromosomes in these regions, he noticed a definite band number decrease, ie, only 67% of the number of bands calculated by Bridges (1938).

In order to confirm or disprove the findings of Berendes (1970),

it would be necessary to re-examine electron microscopically regions 1A-4E of the above chromosome. An investigation such as this, if in agreement with the results of Berendes (1970), would not only contradict the cytological map of Bridges (1938), a dogma that has existed unchallenged for many years, but would also be a further contradiction to the one gene-one band hypothesis of Muller and Prokofyeva (1935). Furthermore, a chromosome band number reduction would be significant in the calculation of DNA within these chromosomes and would, in fact, increase the average band size in terms of haploid DNA content.

It was the purpose of the current investigation then, to repeat the efforts of Berendes (1970) as was suggested above, and also to examine the remaining unobserved regions of the salivary X-chromosome of Drosophila melanogaster as far as possible. Here, then, lies the issue of current investigation, a further challenge of optical systems.

## MATERIALS AND METHODS

Equal numbers of male and female Drosophila melanogaster (Oregon-R, wild type stock) flies were placed/culture bottle. Overcrowding was avoided to insure optimum larval development. Growth media consisted of the conventional corn meal-syrup media and ca. 1-2g powdered brewer's yeast plus a few water droplets added topically. Females were allowed to lay eggs at room temperature for ca. 48 hours, being transferred to fresh media after eggs were observed. Larval development progressed for a period of 7-10 days. Plump, white, late third instar larvae were randomly selected, since sex differentiation was not pertinent to this investigation.

The larvae were placed into fixative and the salivary glands were excised following the method of Demerec and Kaufmann (1967). They were then cleaned of all fatty tissue and each gland cut up into small pieces. Fixation and squashing of the salivary glands were undertaken following a number of methods employing Carnoy's fixative, glutaraldehyde and osmium tetroxide (Berendes and Meyer 1969; Berendes 1970; Ellison 1971; Perov and Chentsov 1971; Sorsa and Sorsa 1967a; Sorsa and Sorsa 1967b; Sorsa 1969a and Sorsa 1969b). However, it was found that a modified Carnoy's solution yielded most consistent results for squashing the salivary glands. In all instances, the aldehyde fixed salivary glands were difficult to squash by hand and a mechanical press was not available for this investigation.

The squashing solution contained a mixture of acetic acid (selected for its good nucleic acid fixation properties), lactic acid (selected as a

tissue softening agent), and distilled water such that the acetic acid concentration remained below 22% (22-a.l.f.). The rationale for this low percentage of acetic acid is that acetic acid in concentrations greater than 22% may cause chromosome interband artifacts (Ellison 1971). The formula consisted of 20.0ml $H_2O$, 11.5ml lactic acid and 8.5ml glacial acetic acid, ie, 21.3% acetic acid.

The total time required to remove, clean and cut up the salivary glands was ca. 2-5 minutes. Apparently this was sufficient for polytene chromosome fixation. It was noticed throughout the study, in using the 22-a.l.f., that a direct relationship existed between the time of exposure to this fixative prior to freezing the squash preparations and the degree of tissue softening that occurred. The lactic acid in the fixative over-softened the tissue if the latter was allowed to remain in fixative in excess of 5 minutes. Figure 2 shows the laboratory area used for squashing and for phase microscopy.

Each 1/2 salivary gland was then placed into a fresh drop of fixative on either siliconized slides or 0.5% plastic coated (araldite, parlodian or clear nail polish), siliconized slides. They were then covered with a clean coverslip and squashed using direct thumb pressure. It was observed that any direct sliding of the coverslip during the squashing usually caused severe chromosome stretching and/or chromosome disorientation. This step in the procedure was a very important variable and perhaps the most critical. The implications of this sliding effect will be discussed in the discussion section of this paper.

Squash preparations were examined with a phase contrast microscope.

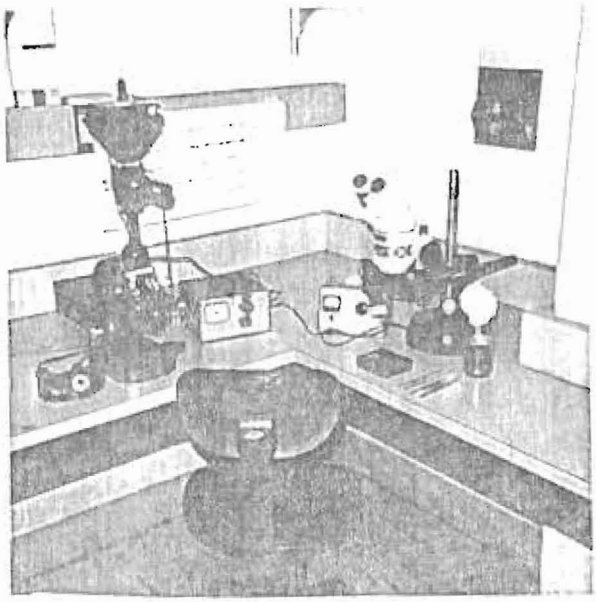

Figure 2. — Salivary glands were removed under disecting microscope (right), squashed and finally observed under the Wild Phase Microscope shown with camera attachment (left).

Phase photomicrographs were taken randomly using the Wild Camera attachment and Kodak Plus-X 35mm film. This procedure was done in the early stages of this investigation for the purpose of chromosome orientation. A high-dry phase photomicrograph is shown in Figure 13. The X-chromosomes were marked with ink scribe directly on the coverslips encircling the general vicinity of the selected chromosome spread. Slides were then marked on the reverse side of the slides with a diamond scribe as directed by the ink mark on the above coverslip. The purpose of this second scribbing was to facilitate locating the desired chromosome after removing the coverslip. Figure 3 shows a dark ink circle (on the coverslip) inside of an etched circle (on the reverse side of the slide).

Slides were then frozen at -186°C. Coverslips were then removed using the edge of a razor blade. Dehydration took place in an acetone-ethanol series and staining in acetone saturated with uranyl acetate (Ellison 1970) (Figure 4). Embedding was performed using modifications of a number of previously described techniques (Gay 1955; Sparivoli, Gay and Kaufmann 1965; Brinkley, Murphy and Richardson 1967; Sorsa and Sorsa 1967c; Berendes 1968 and Ellison 1970). The embedding technique employed small Beem Capsules cut to form cylinders 6-8mm in length. Although the disc method of Ellison (1970) was useful for chromosome identification, it proved to be time consuming and was discontinued. Figure 5 illustrates the disc method of Ellison (1970).

After dehydration and staining took place, a single drop of Araldite-Epon mixture was added to the tissue spread. It was necessary to exhibit speed and accuracy during the addition of the drop of embedding media since

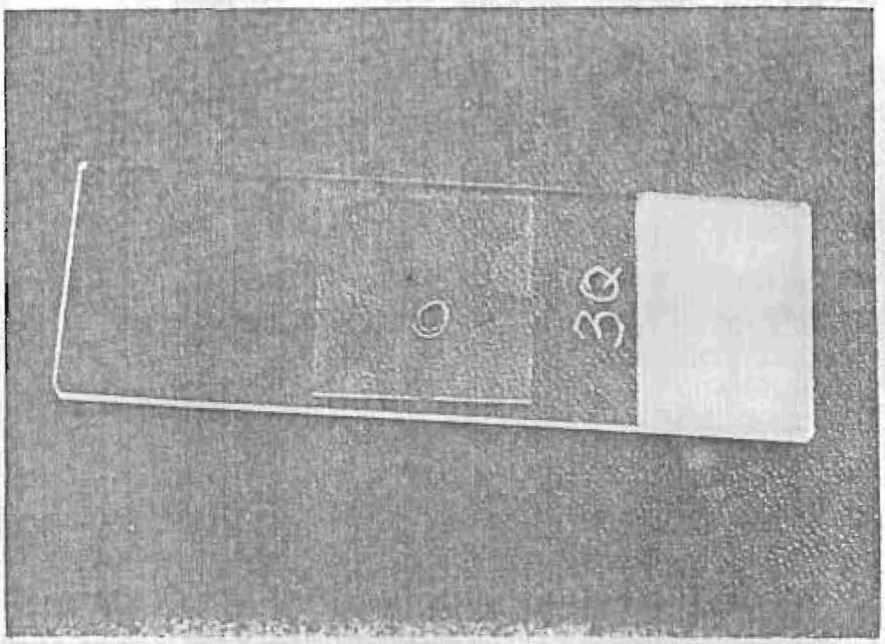


Figure 3. — This figure shows the glass slide covered with an ink marked coverslip. The inner black circle encloses the desired X-chromosome spread. The outer white circle is the diamond scribed mark on the reverse side of the slide seen through the glass.

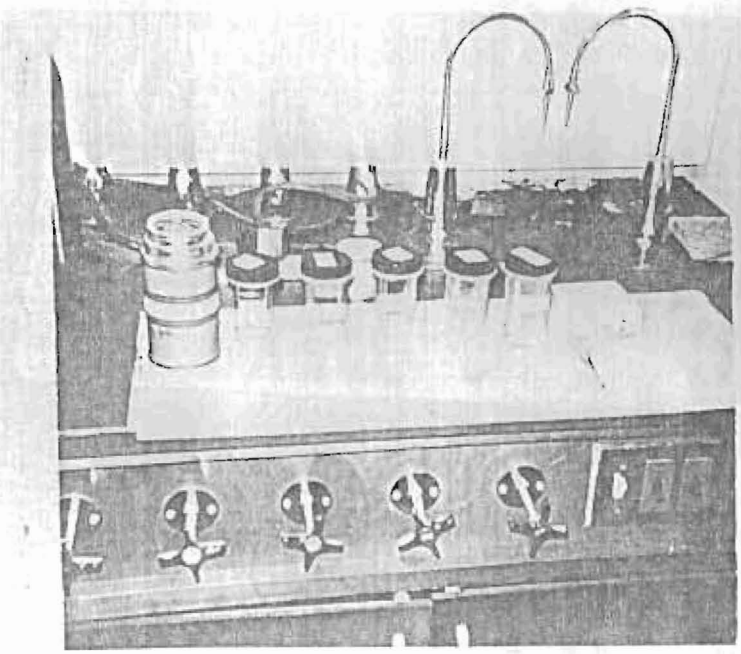

Figure 4. — This figure shows the materials used for freezing, dehydration, staining and embedding the chromosome squash preparations.

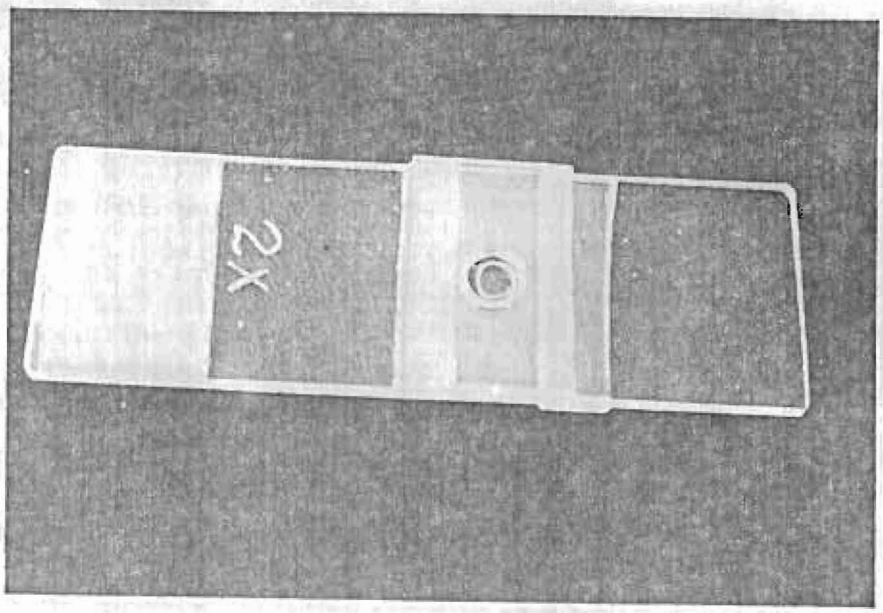


Figure 5. — This figure shows the disc method of Ellison (1970). The small disc is removed from the glass slide after polymerization and must then be glued to a clear cylinder prior to trimming and sectioning.

the acetone saturated tissue spreads dried very rapidly. Any drying at this stage was thought to cause artifacts within the chromosomes. The plastic Beem cylinders were then positioned directly over the drop of embedding media, filled to the brim of the cylinder with this medium and capped with small glass squares (Figures 6 and 7). The embedding medium was prepared either as directed by the manufacturer (Araldite) or as directed by Hayat (1970).

These cylinder assemblies were allowed to polymerize for 48 hours at 59°C. They were then removed from the oven and placed on a hot plate to facilitate the following procedures. Glass squares were first removed from the top of the assemblies followed by removal of the cylinders from the glass slides. The plastic capsule coats were removed using a razor blade or sharp knife. The top of the blocks, identified by small bubbles at their surfaces were marked and designated as that side of the block that did not contain the chromosome spreads.

Blocks were then observed with a phase microscope. X-chromosomes were identified and marked with a crescent (half circle) and pictures were hand drawn for later reference. Using these pictures as guides, blocks were hand trimmed using a sharp razor blade to ca. $1mm^2$ (Figure 8). Extreme care had to be exercised in hand trimming because the blocks became useless if any portions of the X-chromosomes were trimmed away.

Block faces were then fine trimmed on an ultramicrotome to ca. 0.5mm of either square or trapazoid shape. The small size was desired such that later section tearing or buckling would be avoided (Pease 1964; Kay 1967 and Hayat 1970). Although block faces were small, they were, in

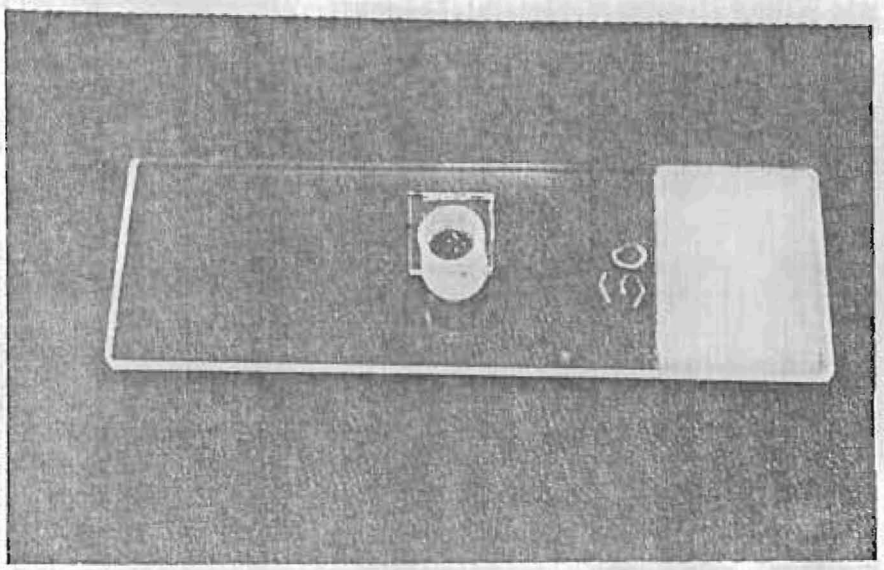

Figure 6. — This modified Beem cylinder proved to be the quickest method. The air bubbles on the surface aid in identification of the chromosome spread after removal from the glass slide. The chromosome would then be on the opposite side of the bubbles since air bubbles always float to the top of the block prior to polymerization.

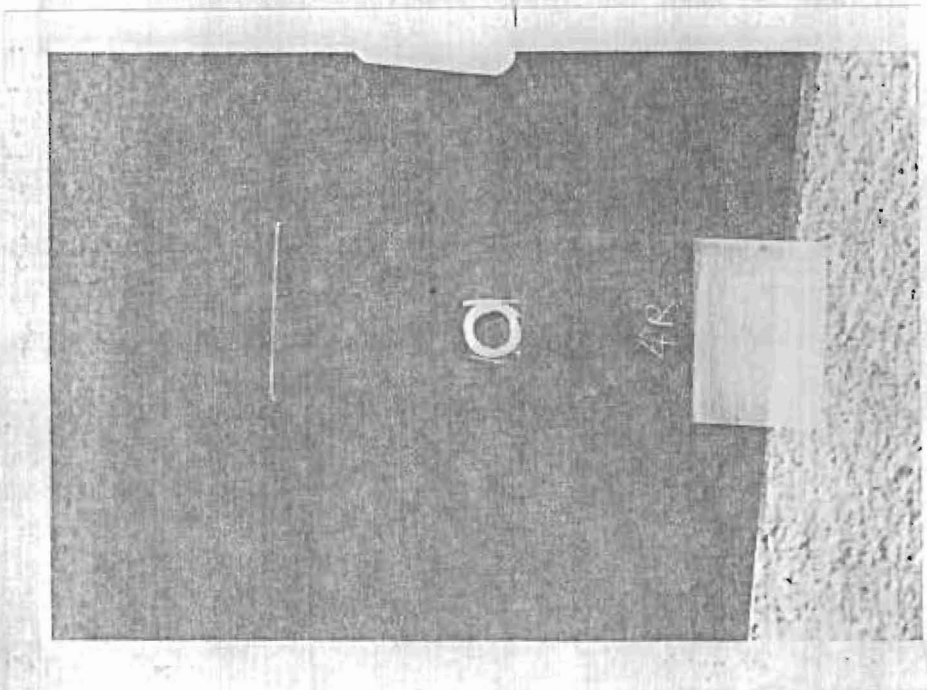


Figure 7. — This figure shows a top surface view of the cylinder assembly as shown in Figure 6.

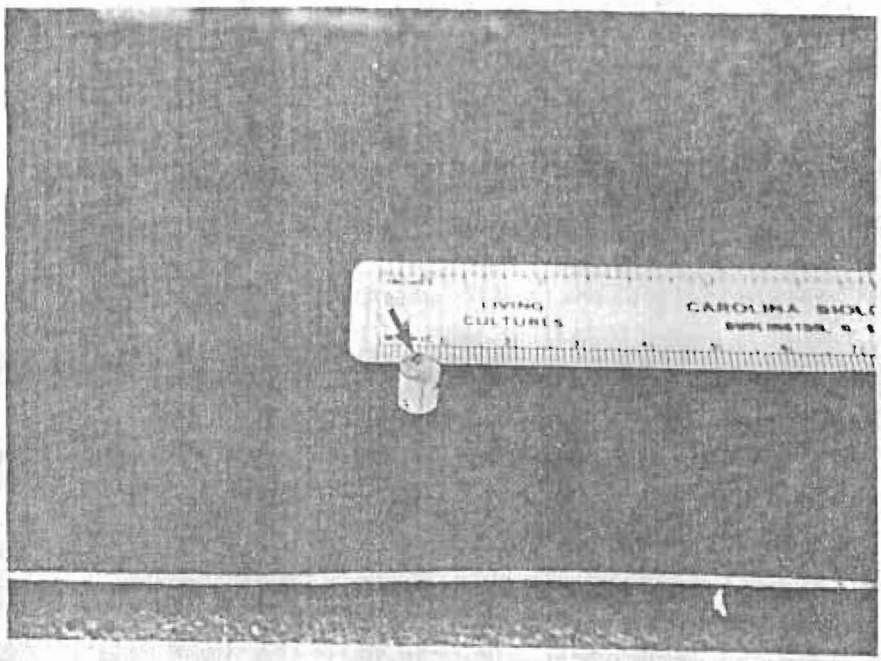


Figure 8. — This figure shows the block after hand trimming prior to fine trimming. Notice the fine ink crescent on the block face (arrow).

fact, quite large in comparison to the X-chromosome and required the aid of a light microscope for identification (Figure 9). The fine trimming of the blocks was accomplished using glass knives prepared either by hand pliers or using the LKB Knife Maker (Figure 10), and trimmed using either the LKB I or III Ultratomes (Figure 11).

Thin sections of ca. 800Å-1500Å (Peachy 1958), ie, silver to dark gold in interference color were obtained using glass knives and sectioned with LKB I, III, Huxley or the Porter-Blum MT-1 ultramicrotomes. Sections were picked up on either collodian (parlodian 0.5% solution) or collodian-carbon stabilized grids. Both the Sjöstrand slot grids and 100 mesh grids were used throughout this investigation.

Serial sections were obtained when possible, but the majority of sections obtained were those first 12-20 sections off of the block face selected at random. Implications of the absence of adequate serial sectioning will be further considered in the discussion section of this paper. All electron microscopy was done at 50kV using either the Hitachi HS-8 or the RCA 3G transmission electron microscopes. The Hitachi HS-8 Electron Microscope is shown in Figure 12. Electron micrographs were prepared using either Kodak D-19 or Dektol developers, Kodak Fixer and Kodabromide F-4 or F-5 high contrast paper using either Omega or Bessler enlargers.

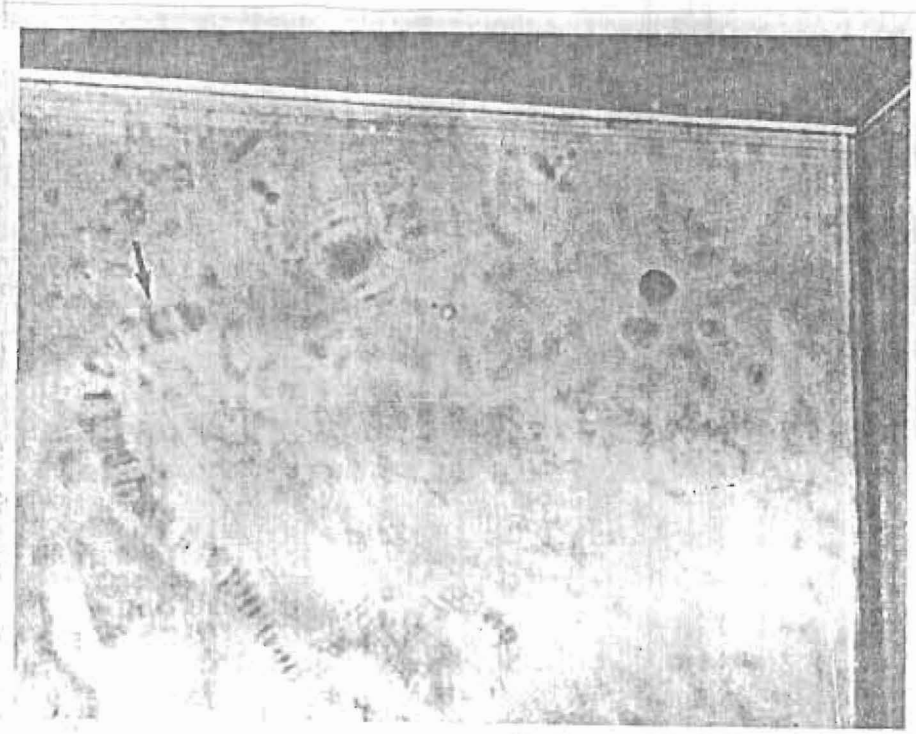

Figure 9. — This figure represents a portion of a phase high-dry photomicrograph showing the X-chromosome (arrow) on the block face prior to sectioning.

Figure 10. — The LKB Knife Maker.

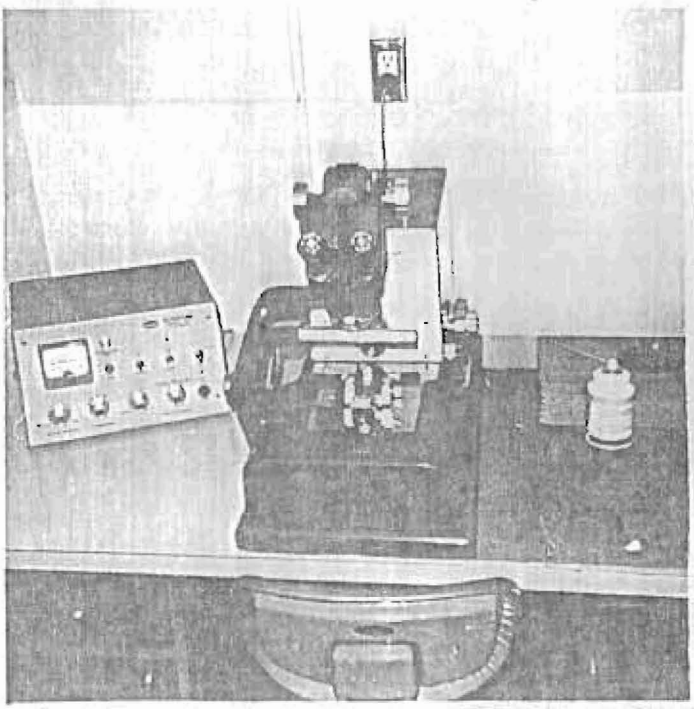

Figure 11. — An ultramicrotome (LKB Ultratome I).

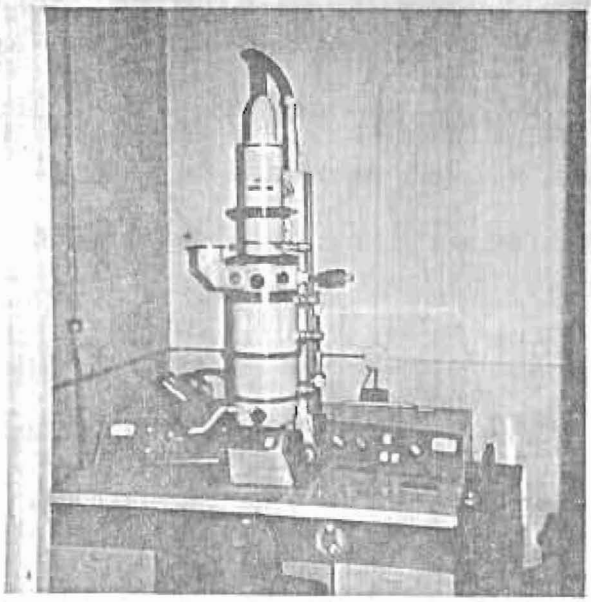

Figure 12. — A small 50kV transmission electron microscope (Hitachi HS-8).

## RESULTS

Comparative results of phase and electron optics are demonstrated in Figures 13 and 14, respectively, showing the first portion (regions 1-7) of the salivary gland X-chromosome of Drosophila melanogaster. The numbers presented in these and all other chromosome figures designate chromosome regions modeled after cytological maps of the X-chromosome of Drosophila melanogaster by Bridges (1935; 1938) and Berendes (1970). In all chromosomes, labeling begins at the X-chromosome tips (region 1) and extends through region 10. Each region is subdivided and thus labeled A through F. These subdivisions were marked according to the maps of either Bridges (1938) or Berendes (1970).

X-chromosome regions studied in the present investigation are designated as 1A through 10F (Figure 15). Further subdivisions of each band were indicated by a subscript, so that the 6 bands found in 1A become $1A_1$ through $1A_6$.

A number of terms are defined in order to avoid later confusion with both tables and figures. A chromosome band will be shown in the following electron micrographs as that nucleoprotein material within the chromosomes that has been bound with the electron dense stain uranyl acetate. Phase photomicrographs are unstained. Apparently, polytene chromosomes do not require staining to observe them under phase optics. As seen in the transmission electron microscope, some bands appeared considerably denser than others and were seen as thick, black areas (Figure 16, Region 1B). Other bands were seen as thinner, less dense

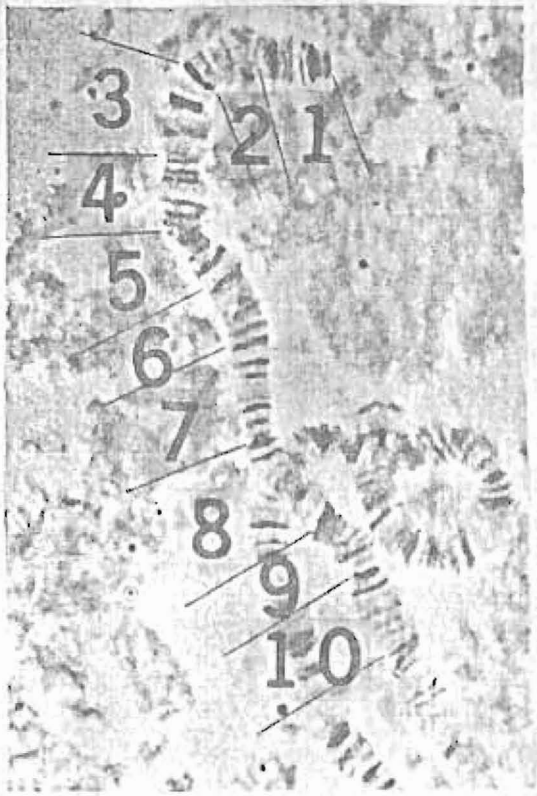



Figure 13 (left). — Unstained high-dry phase photomicrograph of the X-chromosome after squashing on a glass slide. Regions 1-10 marked (ca. 1,000X)..

Figure 14 (right). — Low magnification electron microscopic observation of X-chromosome of Figure 13. Uranyl acetate stained showing Regions 1-7 for comparison (ca. 4,000X).


Figure 15. — This figure shows an electron microscopic representation of the X-chromosome complete from 1A-10F. Arrow indicates a "weak point" (ca. 6,000X).

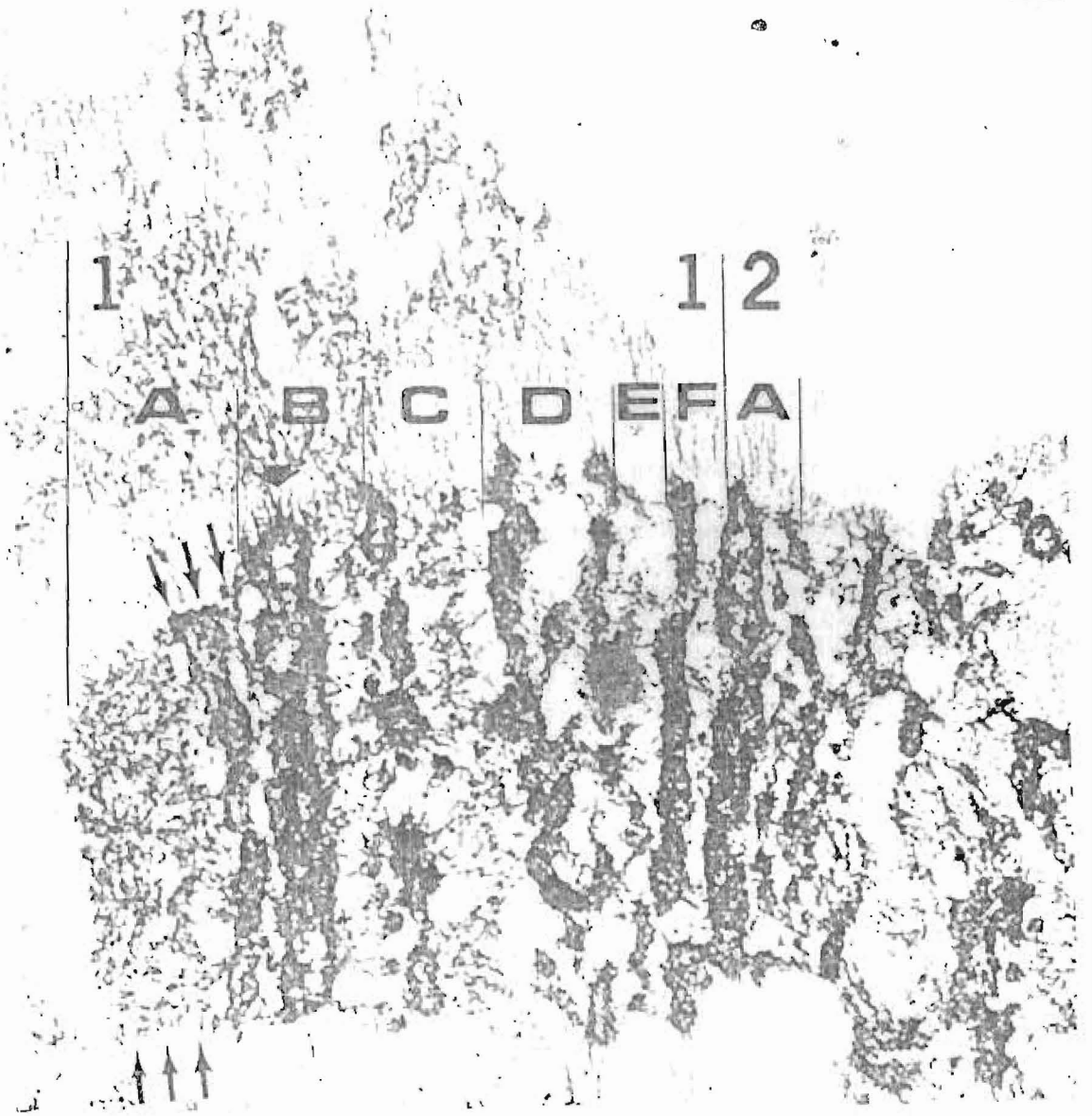


Figure 16. — This is the first of 16 high magnifications of the X-chromosome of the salivary gland of Drosophila melanogaster. The tip of the chromosome here shows Regions 1A-2A. The six arrows shown represent the bands $1A_1$-$1A_6$ marked as close to their "expected" position as possible. The wide arrow shows the thick band of region 1B (ca. 10,000X).

bands (Figure 21, Regions 3E, 3F).

Some bands were also seen as rows of granules and can be visualized and defined as bands (Figure 17, Region 2B). Regions between the banded areas are the interbands and appeared to contain either very fine, linear bands or minute granules (Figure 20, Region 3E). The highly diffuse area in Region 2 of the X-chromosome is the chromosome puff or Balbiani ring (Figures 14, 15 and 17).

In Figures 13, 14 and 15, labeling begins at the top (chromosome tip) and extends downward for the purpose of presenting the entire chromosome portion studied. Figures 16-32 represent higher magnifications of a number of different chromosomes within the regions studied numbered from left to right. This later method aided in establishing correlations with the maps of the earlier investigators (Bridges 1935; 1938 and Berendes 1970).

As will be stressed later, it was quite difficult to determine whether a region was banded or nonbanded due to the high degree of granularity in some regions of the chromosome. In most cases, however, these granular areas possessed a definite linearity; thus band numbers were estimated by counting these rows of linear granules as single bands. Thick bands as well as thinner bands were counted as if they were single bands (singlets) (Figures 23, Region 4F, and 24, Region 6B).

Some bands, especially the thicker ones, appeared as a single band split in half, ergo a double band (doublet). These were counted as two separate bands (Figures 21, Region 3D, and 24, Regions 5C, 6A). The significance of this double feature will become more apparent later in

this paper as it is related to the total numbers of bands counted.

Interband regions generally appeared to be considerably less dense compared to most of the banded areas. Finer bands were often observed within the interbands as well as smaller granules and small fibrils. These small fibrils appeared to exist in a double, parallel configuration. This was observed occassionally in well stretched, highly magnified chromosomes. These fibrils may be the 70Å fibrils described in interband areas of polytene chromosomes by Sorsa and Sorsa (1967b). It was almost impossible to identify these extremely small fibrils in any of the higher magnification micrographs in this investigation due perhaps to the lack of adequate focusing in many of the micrographs.

Those greatly stretched chromosomes observed demonstrate the concept of the protein "backbone" and are highly suggestive of the elastic nature of these polytene chromosomes (Figures 27 and 28) (DuPraw 1970). Some regions of the chromosome exhibited extremely diffuse composition. These regions were observed either at the extreme tip of the chromosome (Figure 16, Region 1A) or within the puff area (Figures 14, 15 and 17, Region 2B). These latter puffs seemed to lack definite band arrangements. Measurements of granule sizes were not accurately calculated but were thought to fall within the range of dimensions described by Berendes (1970), ie, between 250Å-800Å.

Mid-region sections of a total of 10 different X-chromosomes were observed from areas within the Regions 1A through 10F. The tables that follow represent the bands counted in those regions mentioned above. Table 1A and 1B represent the total bands counted of a variety of 10

different X-chromosomes from Regions 1A through 10F.

Notice in Table I that in some regions band counts are not included. The reasons for this was due either to poor clarity in that specific region or that the section obtained did not contain that region of the chromosome. Band numbers in Table I assume that all bands are single. The band numbers shown represent the 10 chromosomes observed in various regions of the chromosome. Notice that this table indicates that there was higher probability of obtaining sections of the chromosome tips than of regions toward the chromosome center.

Table II represents a distribution of those chromosomes containing "probable" double bands (doublets). Thus, bands that appeared split are shown in this table.

Table IIIA compares the electron microscopic observations of Berendes (1970) in Regions 1A through 4E to those observations made in this study in the same regions. Double bands were taken from Table II. Table IIIB completes the observations made in this observation in Regions 4F through 10F. Tables IVA and IVB were included for convenience to compare band numbers between Bridges (1935) and Bridges (1938). Statistical representations of band numbers observed in this study are shown in Table V. Table VI is included as a "hypothetical" map representation of the bands observed in Regions 1A through 10F based on electron microscopic observations. This table is a summation of Tables II and IIIA, B.

Except for Regions 2B (puff region) and Region 7A through 7C (chromosome overlap), all bands included in the map of Table VI were observed in at least two but mostly three different chromosomes.

Calculations of band percentages from electron microscopic observations in Regions 1A through 10F indicate a marked decrease in the total number of bands as observed by Bridges (1938) using the light microscope, ie, 49% of the bands. A greater band number reduction was also observed in Regions 4F through 10F which totaled 41% of the bands of Bridges (1938). Regions 1A through 4E, however, indicate a less marked band number decrease compared to the electron microscopic observations of Berendes (1970) in the above regions. Calculations in Regions 1A through 4E are, in fact, in agreement with Berendes (1970) who calculated 67% of the bands of Bridges (1938) since 66% of the bands of Bridges were observed in these first regions. This overall reduction in the number of bands was thought to have significant implications on the calculation of average DNA per band. The reason for the reduction of observable bands in the regions investigated was thought to be due to the decrease of observable double bands as has been described by Bridges (1938). Further speculation on the reasons for this overall band number reduction will follow in the discussion section of this paper.

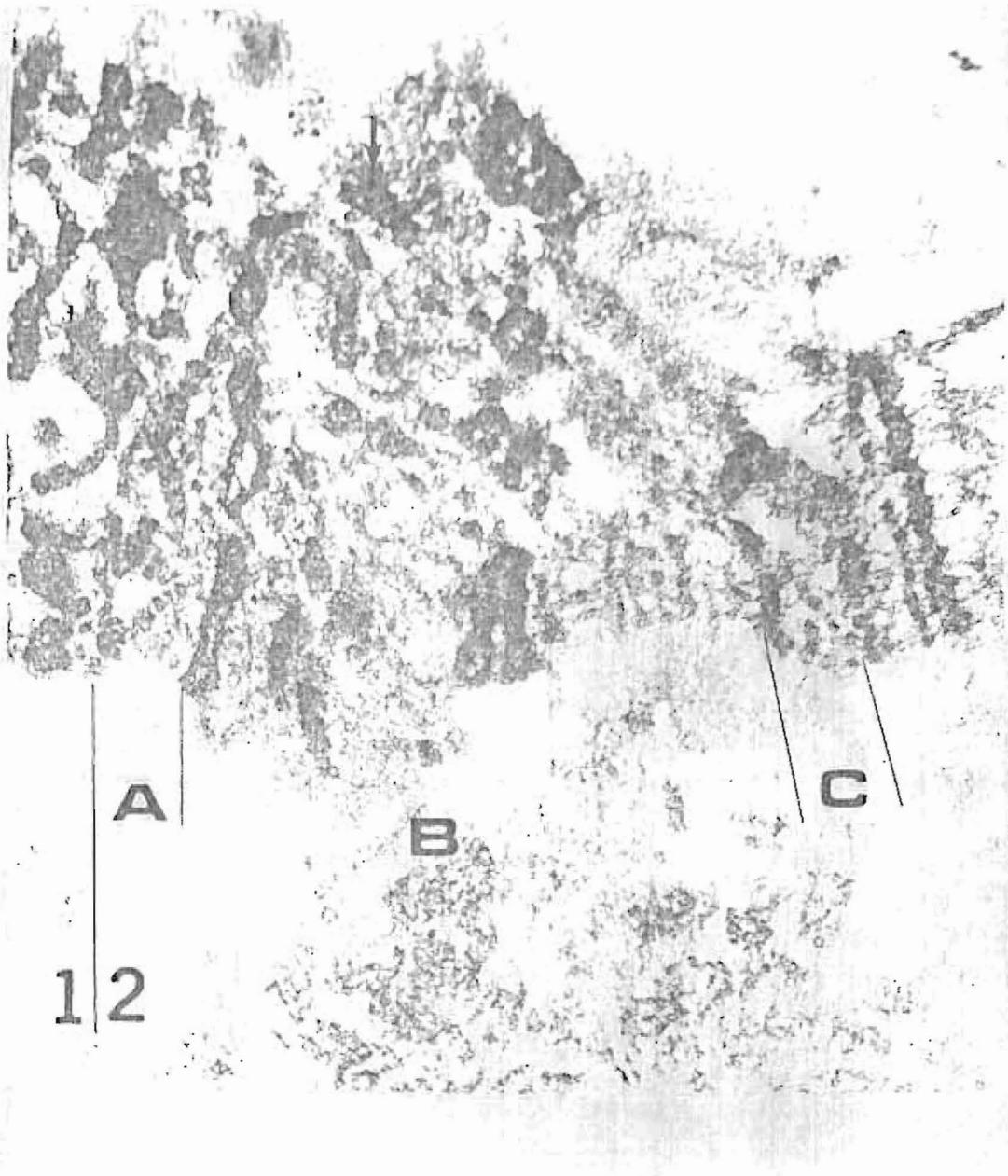


Figure 17. — This figure shows Regions 2A-2C. The chromosome puff is shown in Region 2B. Arrow indicates linear granules which were counted as bands (ca. 11,000X).

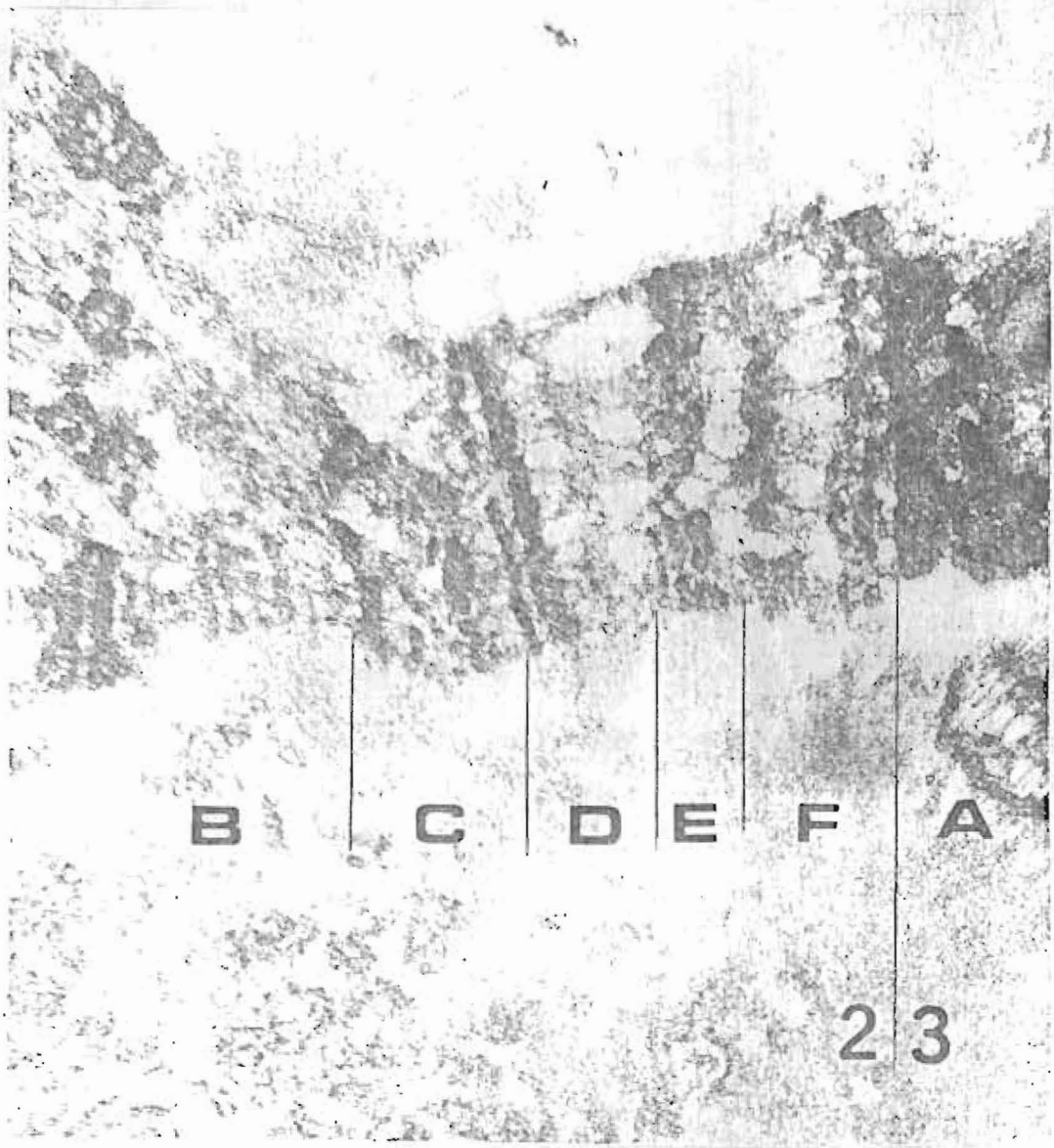


Figure 18. — This figure shows Region 2B-2F (ca. 12,000X).

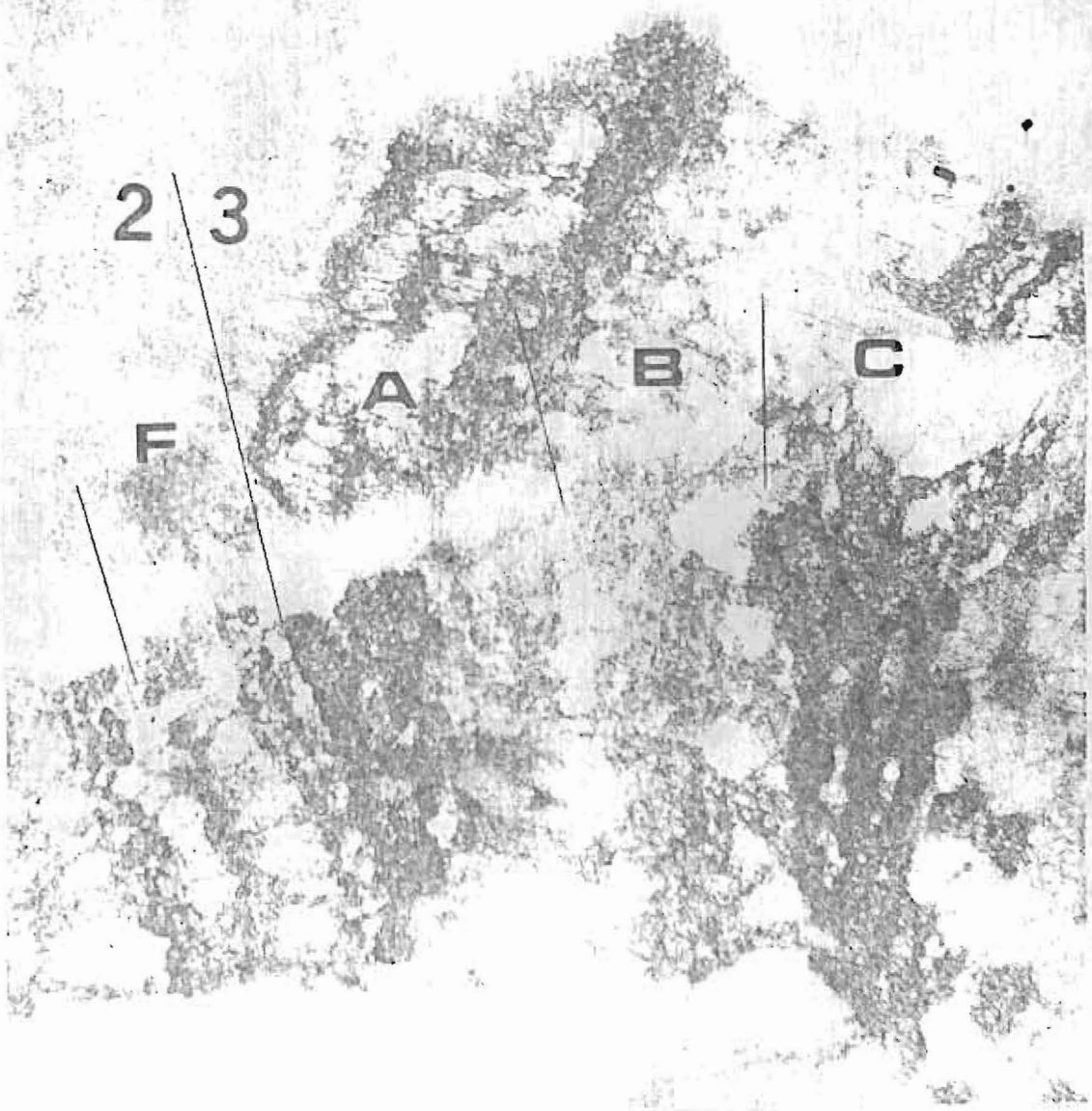


Figure 19. — This figure shows Regions 2F-3B with a portion of the white locus (3C) (ca. 9,000X).

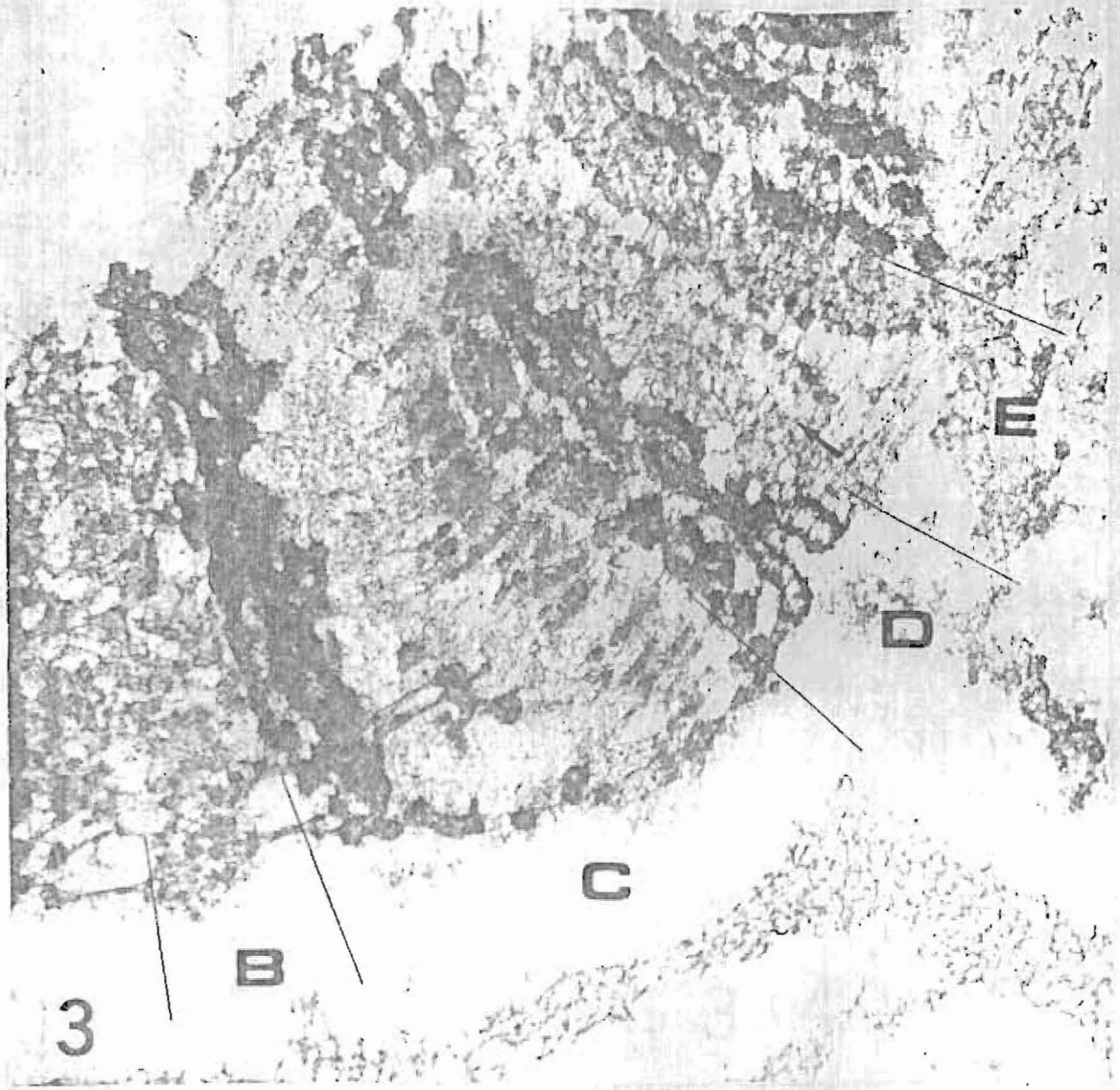


Figure 20. — This figure shows Regions 3B-3E and includes the white locus (3C). Arrow shows minute bands that were seen in some regions as is shown in 3E (ca. 10,000X).

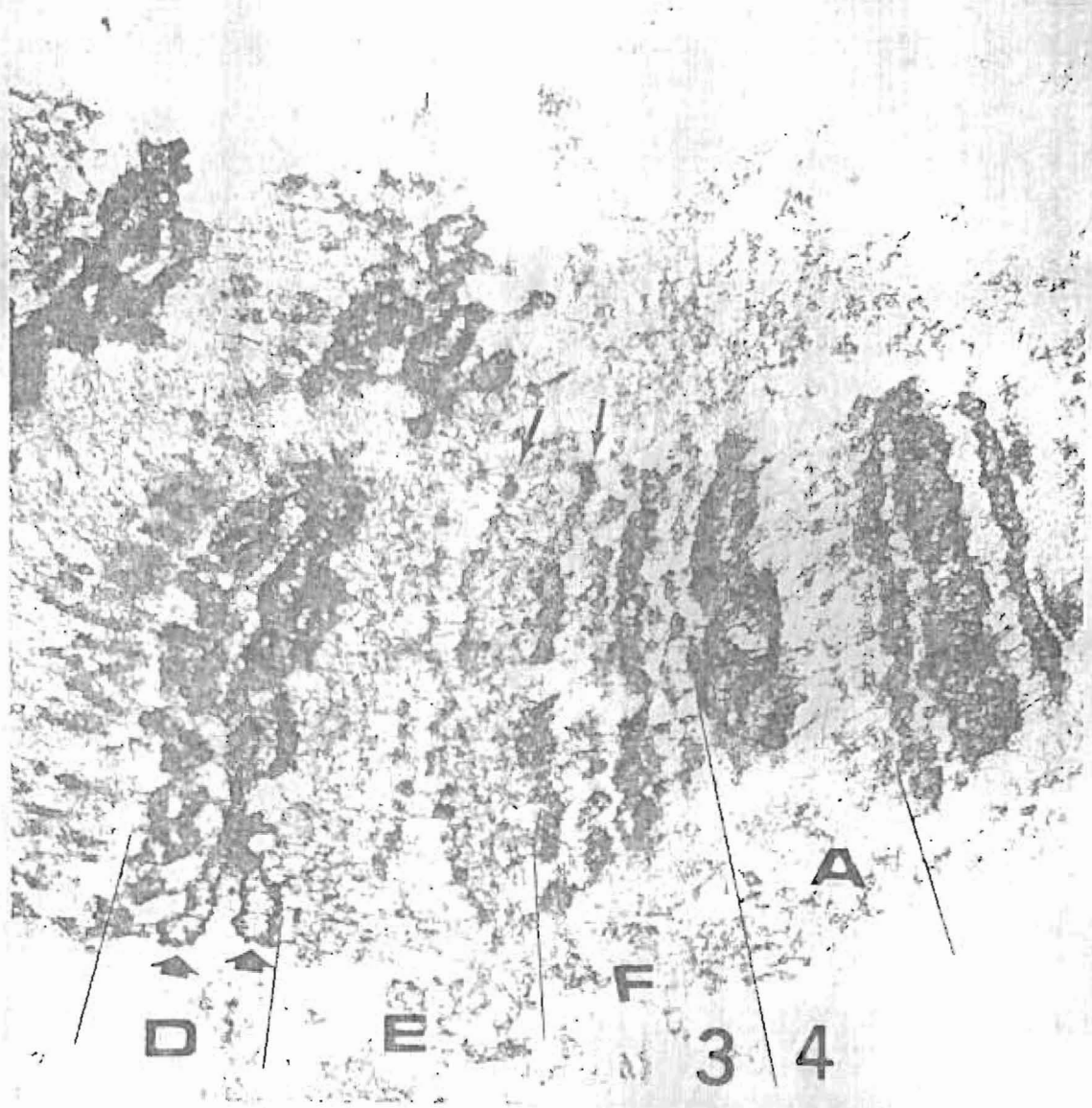


Figure 21. — This figure shows Regions 3D-4A. Thin arrows represents two thinner bands of Regions 3E and 3F. Thick arrow represents two doublets shown in Region 3D (ca. 10,000X).

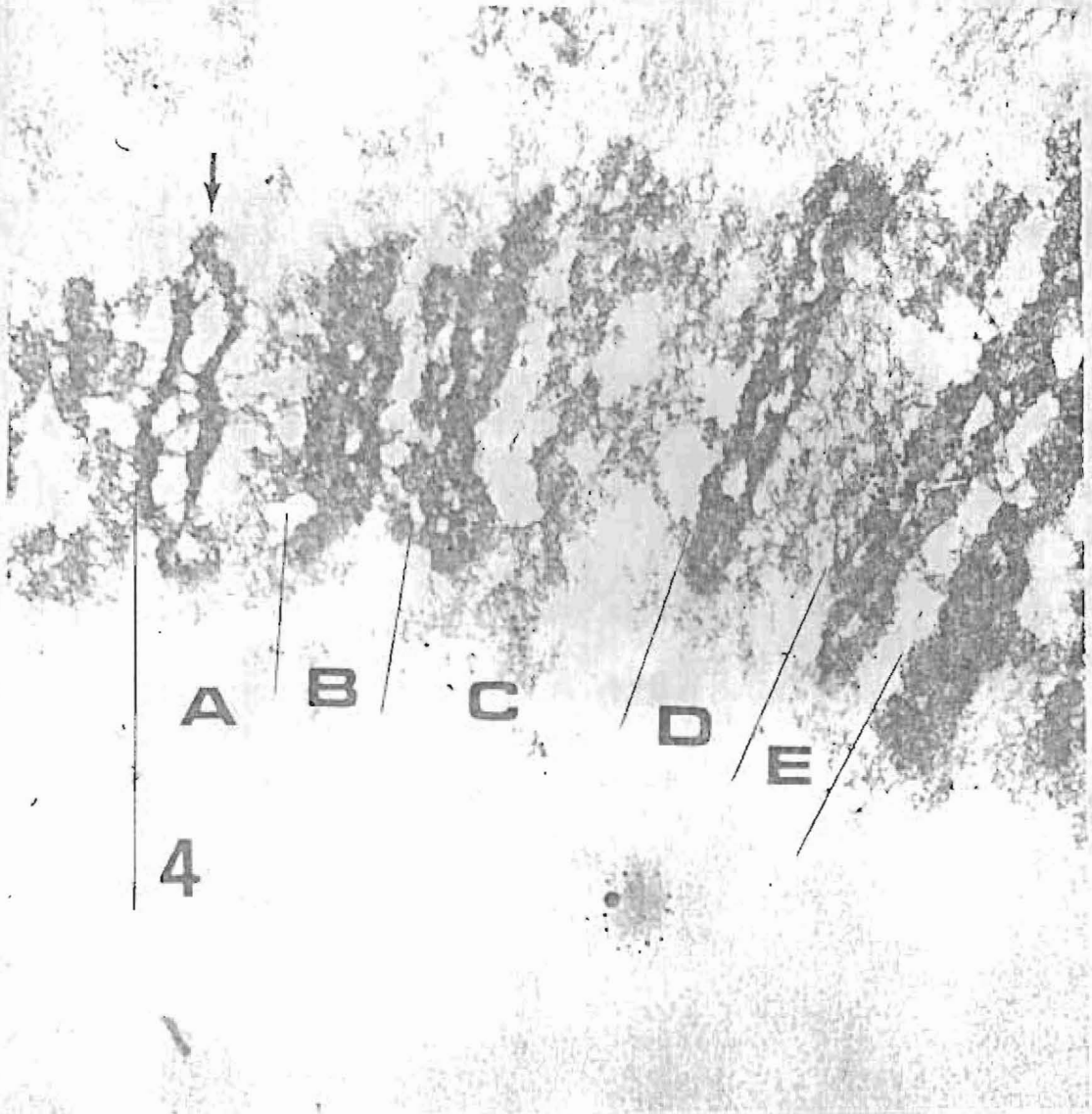


Figure 22. — This figure shows Regions 4A-4E. The arrow shows the acetic acid and/or squashing artifact (ca. 8,000X).

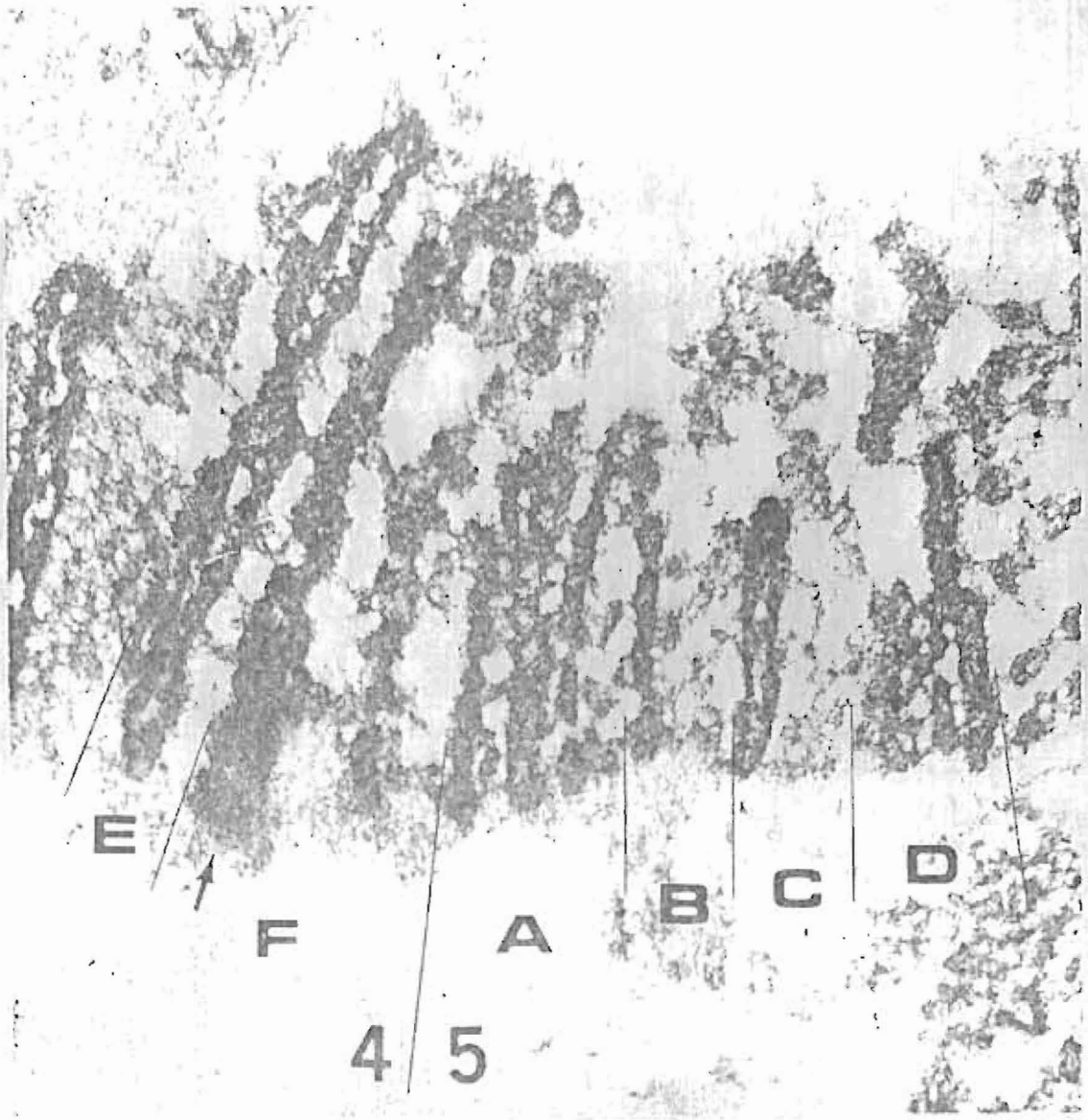


Figure 23. — This figure shows 4E-5D. Arrow shows a thick singlet in Region 4F (ca. 10,000X).


Figure 24. — This figure shows Regions 5A-6B. Arrows show doublet bands in Regions 5C and 6A (ca. 9,000X).

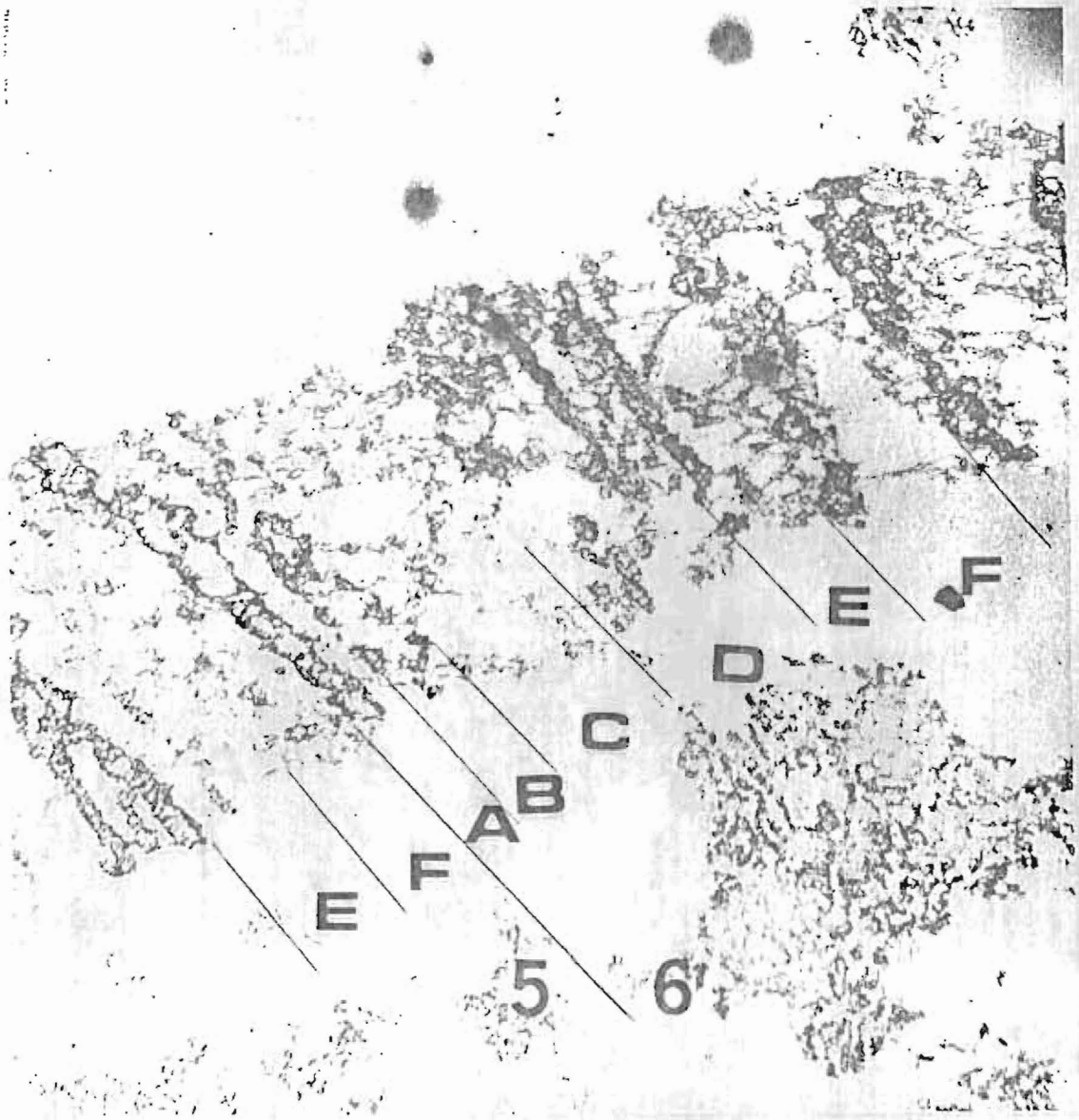


Figure 25. — This figure shows Regions 5E-6F (ca. 8,000X).


Figure 26. — This figure shows two portions of chromosomes overlapping. The horizontal section shows Regions 7A-7D (ca. 8,000X).

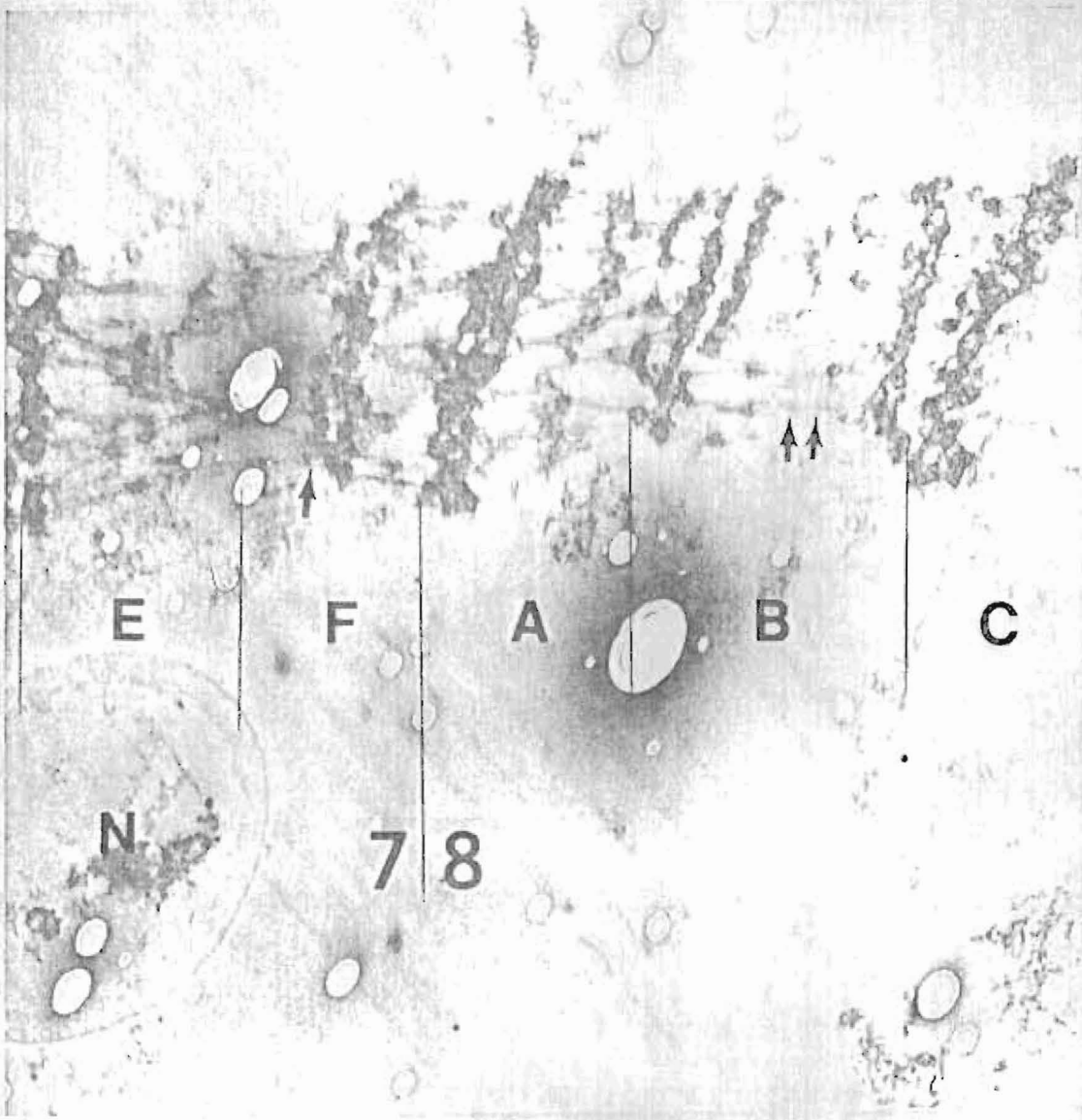


Figure 27. — This figure shows Regions 7E-8C. A portion of a nucleolus is shown as N. Single arrow represents a possible granular band that may have formed as a result of squashing. Double arrow represents high degree of chromosome stretching. The white areas in this micrograph are holes in the parlodian film (ca. 8,000X).

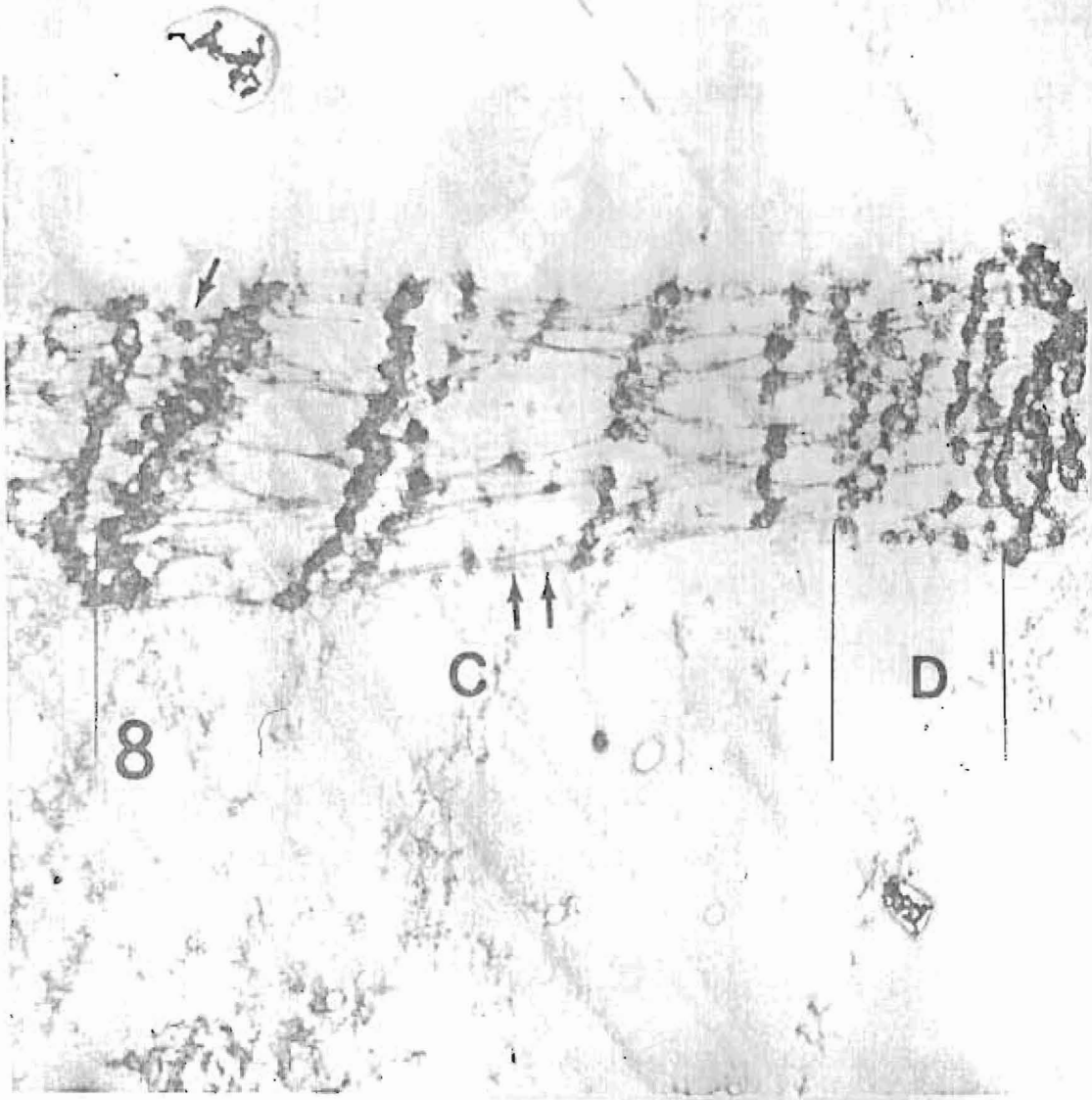


Figure 28. — This figure shows Regions 8C and 8D. Single arrow represents a possible granular band that may have formed as a result of squashing. Double arrow represents high degree of chromosome stretching (ca. 9,000X).

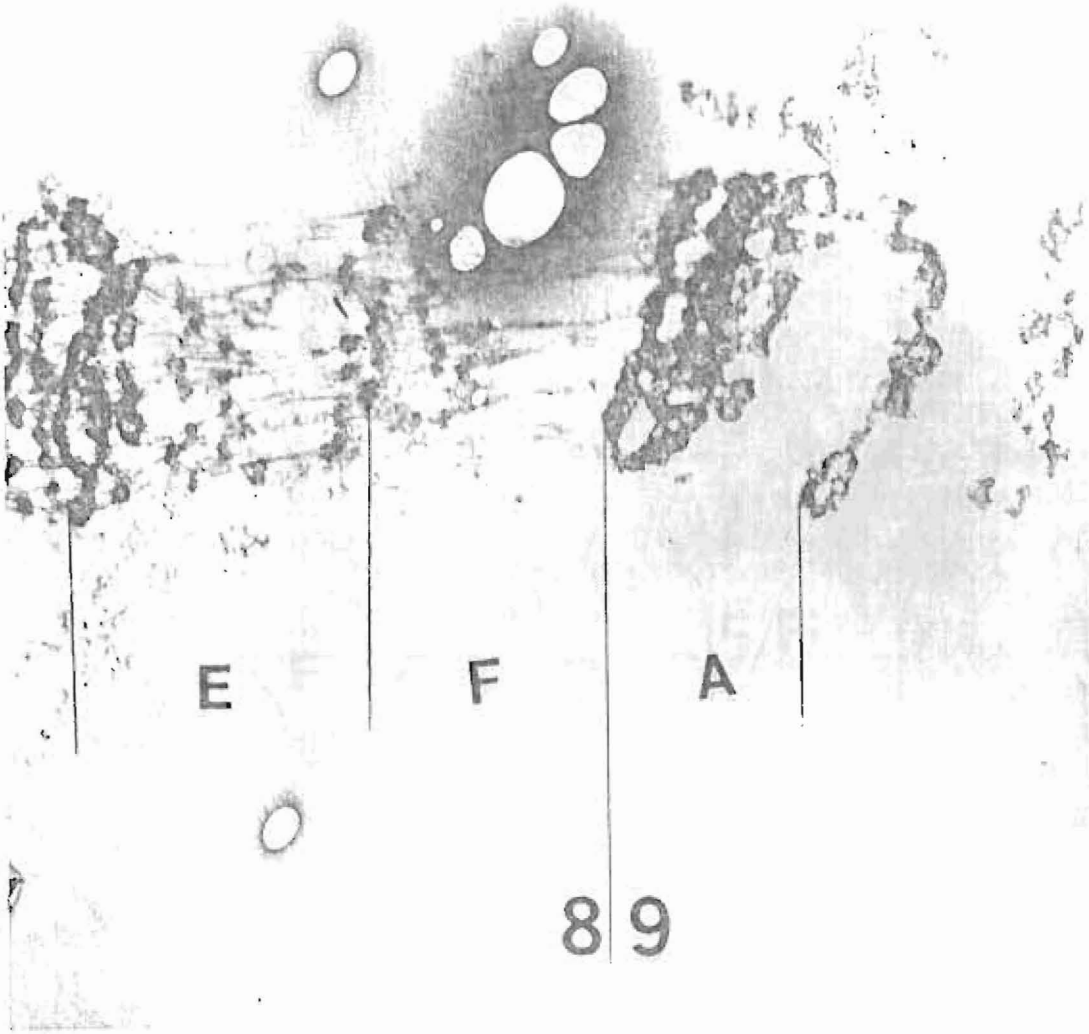


Figure 29. — This figure shows Regions 8E-9A. The white holes shown are artifacts in the parlodian (ca. 8,000X).

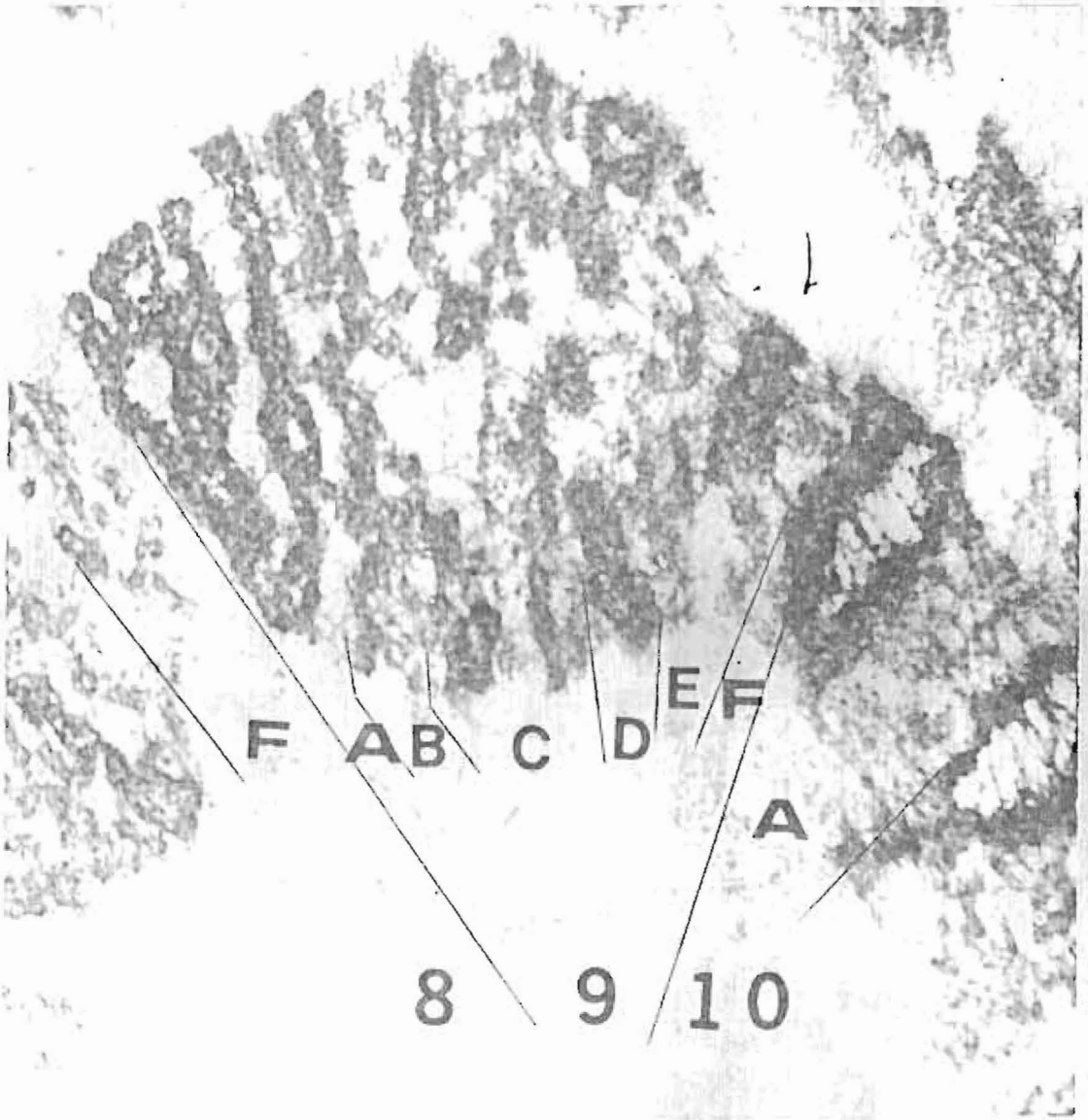


Figure 30. — This figure shows Regions 8F-10A. Notice the band split in 10A. This can be counted as one or two bands and can cause confusion in counting (ca. 8,000X).

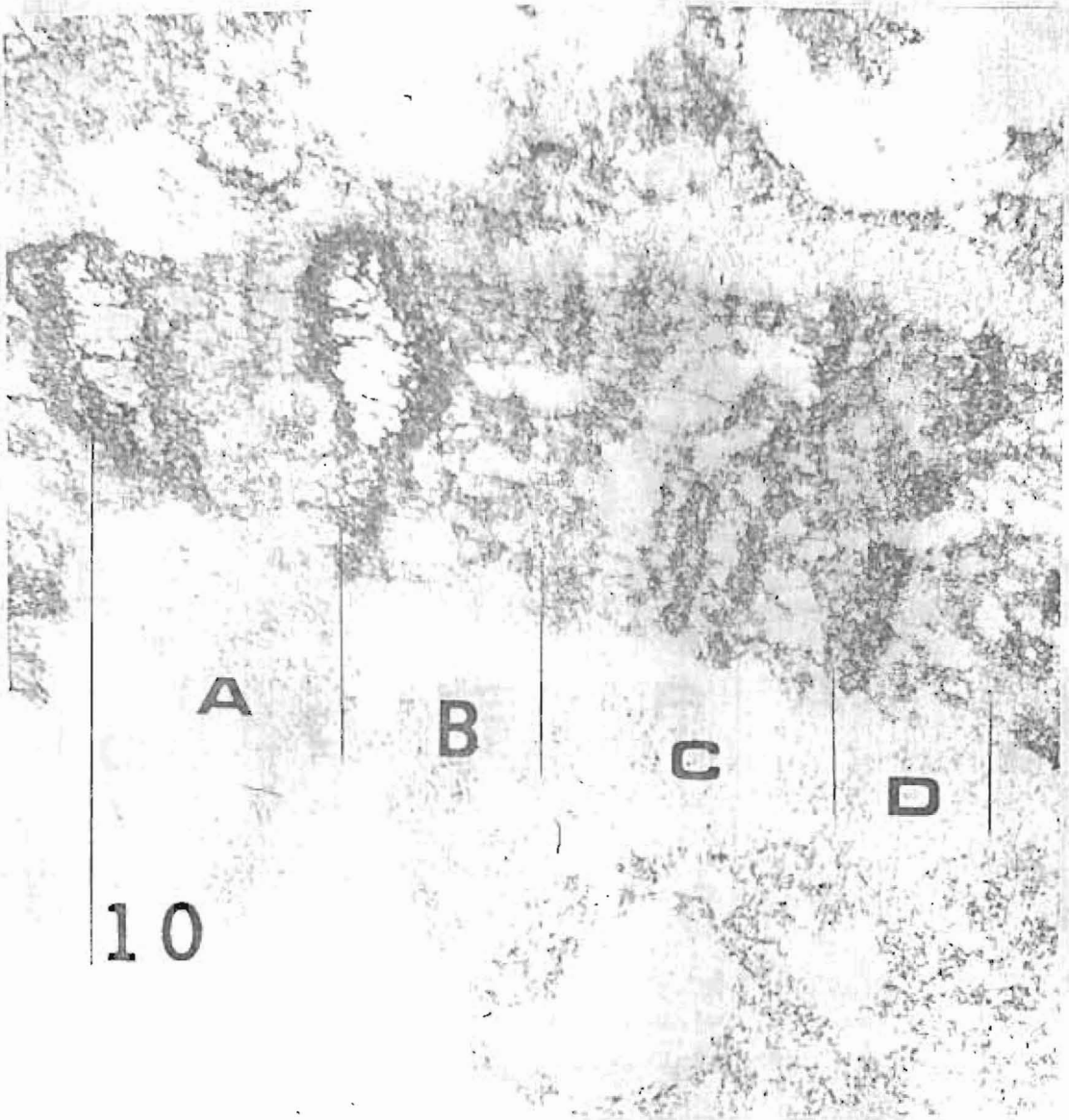


Figure 31. — This figure shows Regions 10A-10C (ca. 8,000X).

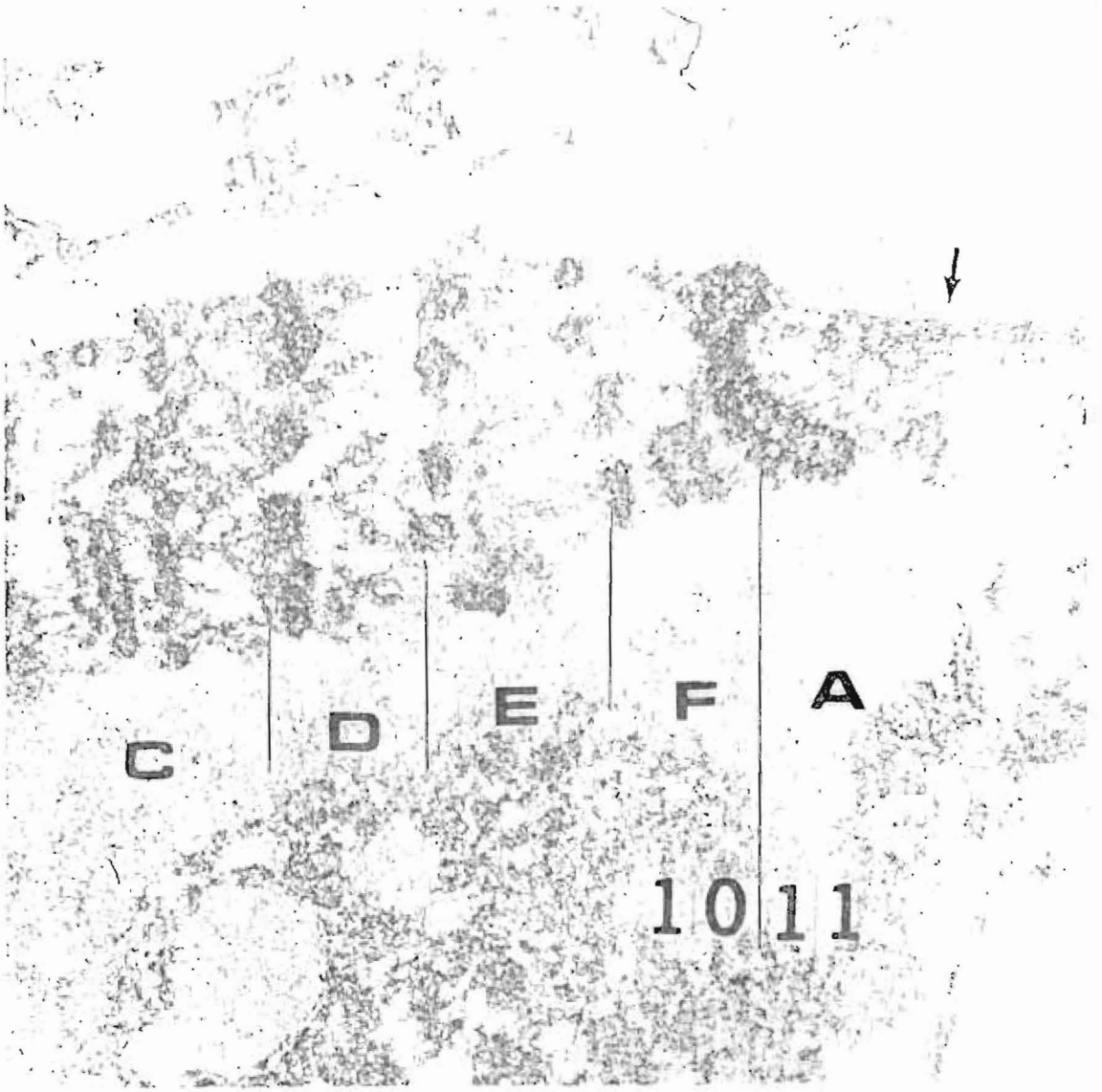


Figure 32. — This figure shows Regions 10C-10F. Arrow shows a "weak point" (ca. 8,000X).

| Chromosome Region | | Chromosomes Sectioned and Observed in the Electron Microscope → 1 | 2 | 3 | 4 | 5 | 6 | 7 | Number of Bands Counted in Each Region ↓ 8 | 9 | 10 | Consistent Band Number per Region ↓ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | - | 3 | 6 | 6 | 4 | - | 5 | 6 | 5 | - | 6 |
| | B | - | 5 | 4 | 4 | 5 | 6 | 6 | 6 | 6 | - | 6 |
| | C | - | 3 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 2 |
| | D | - | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 2 | 3 | 3 |
| | E | - | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | F | - | 4 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 2 | A | 2 | 2 | 3 | 2 | 3 | - | 2 | 2 | 2 | 2 | 2 |
| | B | 6 | 6 | 4 | 8 | 6 | - | - | 15 | - | - | 15* |
| | C | 5 | 3 | 3 | 4 | 5 | 3 | 5 | 3 | 3 | 3 | 3 |
| | D | 2 | 3 | 3 | 2 | 2 | 3 | 2 | 4 | 2 | 3 | 2 |
| | E | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 2 | 2 | 2 | 2 |
| | F | 3 | 3 | 4 | 3 | 3 | 2 | 3 | 2 | 3 | - | 3 |
| 3 | A | 9 | 9 | 9 | 6 | - | 8 | 9 | 9 | - | - | 9 |
| | B | 5 | - | 5 | 4 | - | 5 | 5 | 5 | - | - | 5 |
| | C | 6 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | - | - | 6 |
| | D | 5 | 5 | 6 | 4 | 5 | 4 | 5 | 5 | - | - | 5 |
| | E | 5 | 5 | 5 | 4 | 4 | 5 | 4 | - | - | - | 5 |
| | F | 5 | 3 | 5 | 6 | 6 | 6 | 6 | - | - | - | 6 |
| 4 | A | 3 | 3 | 3 | 3 | 3 | 3 | 3 | - | - | - | 3 |
| | B | 3 | 3 | 3 | 3 | 2 | 3 | 3 | - | - | - | 3 |
| | C | 7 | 7 | 7 | 7 | 7 | 7 | - | - | - | - | 7 |
| | D | 5 | - | 4 | 5 | 4 | 5 | - | - | - | - | 5 |
| | E | 1 | 1 | 2 | 2 | 2 | 2 | - | - | - | - | 2 |

Table I. — (Part A) This first part of Table I is a tabulation of observed data through Regions 1A-4E for the purpose of comparison to Berendes (1970). The continuation of the table is on the following page. *This number of bands in Region 2B was observed only once but introduced into the calculations. No double bands are included in this tabulation.

| Chromosome Region | | Chromosomes Sectioned and Observed in the Electron Microscope → 1 | 2 | 3 | 4 | 5 | 6 | 7 | Number of Bands Counted in Each Region ↓ 8 | 9 | 10 | Consistent Band Number per Region ↓ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | F | 6 | 5 | 5 | 6 | 6 | | | | | | 6 |
| 5 | A | 5 | 5 | 5 | 5 | 5 | | | | | | 5 |
| | B | 3 | 3 | 3 | 3 | 4 | | | | | | 3 |
| | C | 3 | 3 | 2 | 3 | 3 | | | | | | 3 |
| | D | 3 | 3 | 3 | 3 | 3 | | | | | | 3 |
| | E | 4 | 3 | 3 | 4 | 4 | | | | | | 4 |
| | F | 3 | 3 | 2 | 2 | 2 | | | | | | 2 |
| 6 | A | 2 | 2 | 1 | 2 | 2 | | | | | | 2 |
| | B | 2 | 1 | 2 | 2 | 1 | | | | | | 2 |
| | C | 5 | - | 5 | 4 | 5 | | | | | | 5 |
| | D | 3 | 3 | 3 | 2 | 2 | | | | | | 3 |
| | E | 2 | 2 | 3 | 3 | 3 | | | | | | 3 |
| | F | 6 | 3 | 2 | 3 | 3 | | | | | | 3 |
| 7 | A | 4 | 5 | 5 | 5 | 5 | | | | | | 5 |
| | B | 3 | 2 | 2 | - | 2 | | | | | | 2 |
| | C | 6 | 5 | 5 | - | 5 | | | | | | 5 |
| | D | 4 | 4 | 4 | - | 4 | | | | | | 4 |
| | E | - | 5 | 5 | 5 | 5 | | | | | | 5 |
| | F | - | 5 | 5 | 5 | 5 | | | | | | 5 |
| 8 | A | 3 | 2 | 2 | 2 | 2 | | | | | | 2 |
| | B | - | 3 | 3 | 3 | 4 | | | | | | 3 |
| | C | - | 9 | 9 | 8 | 9 | | | | | | 9 |
| | D | - | 4 | 4 | 5 | 4 | | | | | | 4 |
| | E | - | 4 | 4 | 4 | 4 | | | | | | 4 |
| | F | - | 3 | 3 | 3 | 3 | | | | | | 3 |
| 9 | A | - | 5 | 5 | 5 | 5 | | | | | | 5 |
| | B | - | 3 | 3 | 3 | 3 | | | | | | 3 |
| | C | - | 3 | 3 | 2 | - | | | | | | 3 |
| | D | - | 2 | 2 | 1 | - | | | | | | 2 |
| | E | - | 5 | 5 | 5 | - | | | | | | 5 |
| | F | - | 1 | 1 | 1 | - | | | | | | 1 |
| 10 | A | - | 4 | 3 | 3 | - | | | | | | 3 |
| | B | - | 6 | 7 | 6 | - | | | | | | 6 |
| | C | - | 4 | 4 | 4 | - | | | | | | 4 |
| | D | - | 5 | 5 | 5 | - | | | | | | 5 |
| | E | - | 3 | 3 | 4 | - | | | | | | 3 |
| | F | - | 3 | 3 | 4 | - | | | | | | 3 |

Table 1. (Part B) — This is the completion of Table 1 (Part A). Regions 4F-10F are given with data presented in each region of each chromosome. As shown, chromosomes 6-10 were not sectioned through Regions 4F-10E. No double bands are present in this tabulation.

| Chromosome Region | | Chromosomes Sectioned and Observed in the Electron Microscope. Double Bands Observed in Chromosomes → 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Number of Double Bands Counted in Each Region ↓ | Most Consistent Number of Double Bands Observed ↓ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | | | | 1 | | | | | | | | |
| | B | | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | | | 1 |
| | C | | | | 1 | | | | | | | | |
| | E | | | | | 1 | 1 | 1 | 1 | 1 | 1 | | 1 |
| 2 | A | 1 | 1 | 1 | 1 | | | 1 | 1 | | | | 1 |
| 3 | A | 2 | 1 | | | | | | | | | | |
| | D | | | | | 2 | 1 | 2 | | | | | 2 |
| 4 | A | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | 1 |
| | B | 1 | 1 | | 1 | | 1 | | | | | | 1 |
| | C | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | 1 |
| | D | 1 | | 1 | 1 | 1 | 1 | | | | | | 1 |
| | E | 1 | | 1 | 1 | 1 | 2 | | | | | | 1 |
| | F | 1 | 1 | | | | | | | | | | |
| 5 | A | 1 | | | 2 | 2 | | | | | | | 2 |
| | C | 1 | 1 | 1 | 1 | 1 | | | | | | | 1 |
| | D | 1 | | | | 1 | | | | | | | 1 |
| | F | 1 | | | | | | | | | | | |
| 6 | A | 1 | 1 | 1 | 1 | 1 | | | | | | | 1 |
| 7 | A | 1 | 1 | 1 | 2 | 1 | | | | | | | 1 |
| | B | | 1 | 1 | | | | | | | | | 1 |
| | E | | | | 1 | | | | | | | | |
| | F | | | | 1 | | | | | | | | |
| 8 | A | 1 | | | | | | | | | | | |
| | B | 1 | 1 | 1 | 1 | 1 | | | | | | | 1 |
| | C | | | | 1 | | | | | | | | |
| | D | | | | 2 | | | | | | | | |
| | E | | | | | 1 | | | | | | | |
| 9 | A | | 2 | 1 | 1 | 1 | | | | | | | 1 |
| | B | | 2 | | 1 | | | | | | | | |
| 10 | A | | 1 | 1 | 1 | | | | | | | | 1 |
| | B | | 1 | 1 | 1 | | | | | | | | 1 |
| | E | | | | 1 | | | | | | | | |
| | F | | | | 1 | | | | | | | | |
| | | | | | | | | | | | | | 21 |

Table II. — This table gives the distribution of double bands as observed. Doublets were assumed if a band appeared split. These data were used in the map shown in Table IV.

| Chromosomes Region | | Electron Microscopic Observations of Berendes (1970). Single Bands | Double Bands | Granular Areas | Total Bands | Electron Microscopic Observations Granular and Single Bands as one | Double Bands | Total Bands |
|---|---|---|---|---|---|---|---|---|
| 1 | A | 5 | - | 1 | 6 | 6 | - | 6 |
| | B | 6 | (1) | 1 | 8/9 | 4 | 1 | 6 |
| | C | 2 | - | - | 2 | 2 | - | 2 |
| | D | - | 1 | 1 | 3 | 3 | - | 3 |
| | E | - | 1 | 1 | 3 | 1 | 1 | 3 |
| | F | 3 | - | 1 | 4 | 4 | - | 4 |
| 2 | A | 4 | - | - | 4 | 1 | 1 | 3 |
| | B | 14 | - | 1 | 15 | 15 | - | 15 |
| | C | 2 | - | 1 | 3 | 3 | - | 3 |
| | D | 2 | - | 1 | 2 | 2 | - | 2 |
| | E | 2 | 1 | - | 2 | 2 | - | 2 |
| | F | 4 | - | - | 4 | 3 | - | 3 |
| 3 | A | 8 | - | 1 | 9 | 7 | 2 | 9 |
| | B | 4 | - | 1 | 5 | 5 | - | 5 |
| | C | 5 | - | 1 | 6 | 6 | - | 6 |
| | D | 2 | - | 1 | 2 | 3 | 2 | 7 |
| | E | 4 | - | 1 | 5 | 5 | - | 5 |
| | F | 6 | - | - | 6 | 6 | - | 6 |
| 4 | A | 4 | - | - | 4 | 2 | 1 | 4 |
| | B | 3 | - | - | 3 | 2 | 1 | 4 |
| | C | 8 | 1 | 1 | 11 | 6 | 1 | 8 |
| | D | 6 | - | - | 6 | 4 | 1 | 6 |
| | E | 1 | - | 1 | 2 | 1 | 1 | 3 |
| Total Bands | | 94 | 4 | 14 | 116 | 94 | 10 | 114 |

Table III. (Part A) — The above data are tabulations of the band numbers seen in the X-chromosome of Drosophila melanogaster in Regions 1A-4E. Data on the left is from Berendes (1970). Double bands are included in this tabulation (see Table II).

Electron Microscopic Observations

| Chromosome Region | | Granular and Single Bands as one | Double Bands | Total Bands |
|---|---|---|---|---|
| 4 | F | 6 | - | 6 |
| 5 | A | 3 | 2 | 7 |
| | B | 3 | - | 3 |
| | C | 2 | 1 | 4 |
| | D | 2 | 1 | 4 |
| | E | 4 | - | 4 |
| | F | 2 | - | 2 |
| 6 | A | 1 | 1 | 3 |
| | B | 2 | - | 2 |
| | C | 5 | - | 5 |
| | D | 3 | - | 3 |
| | E | 3 | - | 3 |
| | F | 3 | - | 3 |
| 7 | A | 4 | 1 | 6 |
| | B | 1 | 1 | 3 |
| | C | 5 | - | 5 |
| | D | 4 | - | 4 |
| | E | 5 | - | 5 |
| | F | 5 | - | 5 |
| 8 | A | 2 | - | 2 |
| | B | 2 | 1 | 4 |
| | C | 9 | - | 9 |
| | D | 4 | - | 4 |
| | E | 4 | - | 4 |
| | F | 3 | - | 3 |
| 9 | A | 4 | 1 | 6 |
| | B | 3 | - | 3 |
| | C | 3 | - | 3 |
| | D | 2 | - | 2 |
| | E | 5 | - | 5 |
| | F | 1 | - | 1 |
| 10 | A | 2 | 1 | 4 |
| | B | 5 | 1 | 7 |
| | C | 4 | - | 4 |
| | D | 5 | - | 5 |
| | E | 3 | - | 3 |
| | F | 3 | - | 3 |
| Total Bands | | 127 | 11 | 149 |

Table III. (Part B) — The above tabulation represents electron microscopic observations in Regions 4F-10F. Double bands are included.

| Chromosome Region | Map of Bridges (1935) Single Bands | Double Bands | Total Bands | Map of Bridges (1938) Single Bands | Double Bands | Total Bands |
|---|---|---|---|---|---|---|
| 1 A | 3 | 2 | 7 | 6 | 1 | 8 |
| B | 7 | - | 7 | 6 | 4 | 14 |
| C | 3 | - | 3 | 1 | 2 | 5 |
| D | 1 | 1 | 3 | 2 | 1 | 4 |
| E | 2 | 1 | 4 | 1 | 2 | 5 |
| F | 1 | 1 | 3 | 2 | 1 | 4 |
| 2 A | 1 | 1 | 3 | - | 2 | 4 |
| B | 8 | - | 8 | 6 | 6 | 18 |
| C | 3 | 1 | 5 | 4 | 3 | 10 |
| D | 6 | - | 5 | 2 | 2 | 6 |
| E | 2 | - | 2 | 1 | 1 | 3 |
| F | 4 | - | 4 | 6 | - | 6 |
| 3 A | 5 | 2 | 9 | 8 | 1 | 10 |
| B | 3 | - | 3 | 2 | 1 | 4 |
| C | 6 | 2 | 10 | 4 | 4 | 12 |
| D | 1 | 2 | 5 | 4 | 1 | 6 |
| E | 2 | 1 | 4 | 2 | 3 | 8 |
| F | 4 | - | 4 | 3 | 3 | 9 |
| 4 A | 4 | - | 4 | 2 | 2 | 6 |
| B | 3 | - | 3 | 2 | 2 | 6 |
| C | 5 | 2 | 9 | - | 8 | 16 |
| D | 5 | - | 5 | 3 | 2 | 7 |
| E | 3 | - | 3 | 1 | 1 | 3 |
| Total Bands | 82 | 16 | 114 | 68 | 53 | 174 |

Table IV. (Part A) — The data above was taken from the maps of the X-chromosome of *Drosophila melanogaster* from Bridges (1935) on the left and from Bridges (1938) on the right. They show data in Regions 1A-4E for comparison to the electron microscopic map of Berendes (1970).

| Chromosome Region | | Map of Bridges (1935) Single Bands | Double Bands | Total Bands | Map of Bridges (1938) Single Bands | Double Bands | Total Bands |
|---|---|---|---|---|---|---|---|
| 4 | F | 4 | 1 | 6 | - | 7 | 14 |
| 5 | A | 3 | 1 | 5 | 6 | 4 | 14 |
| | B | 3 | - | 3 | 2 | 4 | 10 |
| | C | 6 | - | 6 | 4 | 3 | 10 |
| | D | 3 | 2 | 7 | - | 4 | 8 |
| | E | 4 | - | 4 | 2 | 3 | 8 |
| | F | 2 | - | 2 | 2 | 2 | 6 |
| 6 | A | 4 | - | 4 | - | 2 | 4 |
| | B | 1 | 1 | 3 | - | 2 | 4 |
| | C | 3 | - | 3 | 7 | 3 | 13 |
| | D | 2 | - | 2 | 2 | 3 | 8 |
| | E | 3 | - | 3 | 2 | 2 | 6 |
| | F | 5 | - | 5 | 1 | 5 | 11 |
| 7 | A | 4 | - | 6 | 2 | 3 | 8 |
| | B | 4 | - | 4 | 6 | 1 | 8 |
| | C | 5 | - | 5 | 3 | 3 | 9 |
| | D | 8 | - | 8 | 8 | 7 | 22 |
| | E | 6 | - | 6 | 5 | 3 | 11 |
| | F | 4 | 1 | 6 | 4 | 3 | 10 |
| 8 | A | 1 | 1 | 3 | 1 | 2 | 5 |
| | B | 4 | 2 | 8 | 4 | 2 | 8 |
| | C | 11 | - | 11 | 7 | 5 | 17 |
| | D | 5 | - | 5 | 4 | 4 | 12 |
| | E | 7 | - | 7 | 2 | 5 | 12 |
| | F | 4 | - | 4 | 2 | 4 | 10 |
| 9 | A | 5 | - | 5 | 5 | - | 5 |
| | B | 6 | 1 | 8 | 1 | 7 | 15 |
| | C | 2 | 1 | 4 | - | 3 | 6 |
| | D | 3 | - | 3 | 2 | 1 | 6 |
| | E | 6 | - | 6 | 2 | 4 | 10 |
| | F | 5 | - | 5 | 5 | 4 | 13 |
| 10 | A | 8 | - | 8 | 1 | 5 | 11 |
| | B | 11 | - | 11 | 5 | 6 | 17 |
| | C | 4 | 1 | 6 | - | 5 | 10 |
| | D | 5 | - | 5 | 6 | 1 | 8 |
| | E | 5 | - | 5 | 2 | 2 | 6 |
| | F | 5 | - | 5 | 5 | 3 | 11 |
| Total Bands | | 161 | 13 | 187 | 110 | 127 | 364 |

Table IV. (Part B) — The above tabulation is the completion of Bridges (1935) on the left and Bridges (1938) map on the right. Regions shown here are 4F-10F. The totals of Regions 4F-10F in Bridges (1935) map were counted as closely as possible since no values were given as to single or double bands.

| Investigator | Region Investigated | Total Band Number Counted | Percentage Calculations |
|---|---|---|---|
| Bridges (1938 Light microscopic | 1A-4E | 174 | ——— |
| Berendes (1970) Electron microscopic | 1A-4E | 116 | 67% of Bridges (1938) map |
| Electron microscopic observations | 1A-4E | 106 as single bands 114 with doublets | 61% of Bridges (singlets). 66% assuming doublets. 98% of Berendes (1970). |
| Bridges (1938 Light microscopic | 4F-10F | 364 | ——— |
| Electron microscopic observations | 4F-10F | 138 as single bands 149 with doublets | 38% of Bridges (singlets). 41% assuming doublets |
| Bridges (1938) Light microscopic | 1A-10F | 538 | ——— |
| Electron microscopic observations | 1A-10F | 263 with doublets | 49% of Bridges (1938) |

Table V. — The above Table is a tabulation of Tables I through III showing percentage calculations.

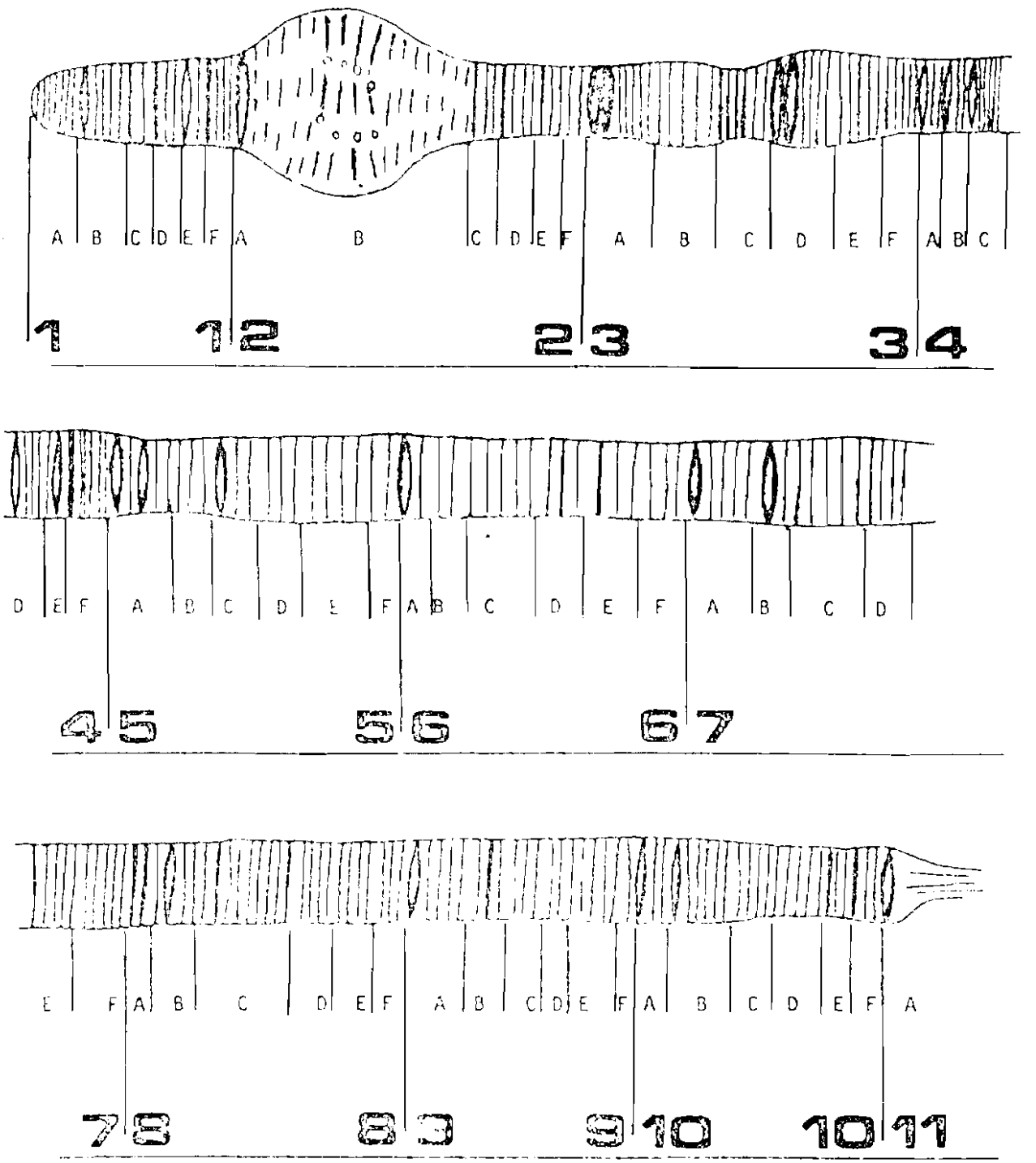


Table VI. — This table represents a map of Regions 1A-10F of the X-chromosome of Drosophila melanogaster taken from electron micrographs in this first half of the chromosome. Lines drawn representing bands are either thin singlets, thick singlets, granular singlet areas (puff 2B), or doublets (ca. 5,000X).

# DISCUSSION

The polytene chromosomes have been the prototypes for genetical and cytological studies for many years. They have been thoroughly investigated with the light microscope and have only recently been studied using electron optics. Early investigations of the polytene chromosomes began in the late 1940's and early 1950's but the results were as inferior as the techniques employed. In these studies, whole salivary glands were sectioned, as flat embedding techniques were not available in the literature.

Herskowitz (1952), however, managed to describe large numbers of granular particles within the interband regions in electron micrographs of acetic acid fixed chromosomes. Heilweil, Heilweil and van Winkle (1952) also confirmed the presence of granular particles that had diameters of 300Å using similar methods. Loman (1956) used Zenker's Fixative and also described these particles. Borysko (1953) noticed a depolymerization of nucleic acid and a general disorganization of bands after fixing the salivary glands in osmium tetroxide.

The choice of fixative is important in the study of polytene chromosomes both at the light and electron microscopic levels. Acetic acid, a good fixing agent for nucleoproteins has been the choice fixative for many years. It has been used in various fixing formulae by earlier investigators using the light microscope (Painter 1934a; Bridges 1935; 1938), and by a number of electron microscopists investigating the polytenes both early (Herskowitz 1952; Loman 1956), and more recently (Berendes 1968; Sorsa and Sorsa 1967b; 1967c; Sorsa, Pusa, Virrankoski and Sorsa 1970; Sorsa 1969c;

Berendes 1970; Ellison 1971 and others).

Acetic acid has been known to cause certain artifacts, especially within the bands, artifacts that may be highly significant in cytological map preparations. Herskowitz (1952) suggested that it is important to consider the physio-chemical nature of the polytene chromosomes for any satisfactory interpretation of chromosome structure. He suggested that there is a loss of water and glycogen from the chromosome upon exposure to acetic acid. It has also been demonstrated by Kurnick and Herskowitz (1952) that desoxyribonucleohistone is transformed into precipitated sheets and fibers after acetic acid exposure, thus indicating that definite physical changes occur as well. Acetic acid is also thought to cause chromosome shrinkage (Herskowitz 1952).

Sorsa and Sorsa (1967b) suggested that acetic acid destroyed the membranous fine structure of the cytoplasm. Sorsa (1969c) demonstrated a high degree of band vacuolization using 45% acetic acid fixation. This was compared to a 3:1 acetic acid-methanol fixative which produced considerably less vacuolization.

Despite these disadvantages, however, acetic acid has been employed in electron microscopic investigations since it offers good contrast when combined with the heavy metal uranyl acetate (Sorsa and Sorsa 1967b). Ellison (1971) suggested that acetic acid used in concentrations less than 22% would not produce significant artifacts. A concentration of 21.3% used in this investigation apparently did produce this condition, however, in a number of different chromosomes (Figures 22 and 24). This artifact is significant since it relates to the doublet band formation.

Other fixatives were met with some opposition. All aldehydes employed made the chromosomes very hard and difficult to squash under normal thumb pressure. (Foot presses are generally used by others to squash the salivary glands and can produce up to 3,000 lbs. pressure). This was another reason for the selection of acetic acid as the fixative used in this study. Osmium tetroxide does not produce adequate fixation and was not used. The acetic acid precipitates nucleoproteins quite readily forming a high degree of compactness as seen in the chromosome figures.

Berendes (1968) compared fixatives in an early study of the salivary gland X-chromosome of Drosophila melanogaster. He compared both aldehyde and acetic acid fixatives and squashed all salivary glands in 40% acetic acid. Interestingly enough, banding patterns were found to be similar in all pre-fixed chromosomes. The artifacts described in the present investigation tend to be in agreement with the artifacts discussed by Sorsa (1969c).

Recent electron microscopists have investigated other arms of the Drosophila polytenes as well as those in a number of other animals and plants. Perov and Chentov (1971) described a high degree of disc structures in Chironomus salivary gland chromosomes. Sorsa and Sorsa (1968a) investigated the salivary gland polytenes of Drosophila melanogaster describing granular regions of the bands as representing "small individual chromomeres." Many chromomeres were described to be connected by short, longitudinal fibrils resembling the interband fibrils. High magnifications of the interband regions showed that they were composed of parallel fibrils,

oftened arranged in loops or coils. These fibrils were determined to be 70Å-150Å in diameter. Each of these fibrils have been further resolved to be composed of two smaller, parallel subfibrils of 20Å-30Å in diameter, ie, the approximate dimensions of the DNA molecule. The parallel configuration of the interband fibrils was observed on one occasion using the high magnification pole piece of the Hitachi HS-8 microscope and were probably the larger 70Å fibrils mentioned.

Sorsa and Sorsa (1968a) also suggested that the squashing pressure separates the bands into smaller bands indicating that the bands may be composed of several successive chromomeres (Figures 27 and 28). Other thicker bands seem to retain their integrity (Figures 18 and 23). This may have some genetical significance if a structural difference exists to cause this difference in band appearance. This could also have some bearing on certain types of chromosomal breaks as in interchromomeric crossing over.

Sorsa and Sorsa (1968a) suggest that the band compactness is due to the accumulation of "primary gene product" and protein-like substances in that band region. The puffing pattern of these polytenes has also been described by Sorsa (1969b) who described high granularity and fibrous appearance in these specific sites along the chromosomes. In most cases, banding patterns within these puffing regions are very difficult to interpret. Berendes (1970) showed 15 bands in this region and although this number was only observed in one chromosome in this study, it was used in the calculation of percentages. Sorsa and Sorsa (1969b) speculated that this puff disorganization is attributable to the high amount of

matrix substances accumulating during the synthesis of RNA.

With reference to the interbands of the polytenes, Sorsa and Sorsa (1968b) described small 100Å-200Å granules within the interband regions situated in rows longitudinally oriented. These particles were speculated to connect two adjacent 30Å fibrils together suggesting some role as replication guides between the parallel 30Å DNA units. These authors also mention the term "miniband" which forms when the chromomeres are highly stretched.

These general electron microscopic observations also apply to the salivary gland X-chromosome of Drosophila melanogaster as shown by Berendes (1968; 1970) and as described from electron micrographs in this investigation. From the observations in both plants and animal polytenes, very elaborate chromosome models have been prepared (Loman 1955; Sorsa and Sorsa 1967b; 1967c; 1968b and others).

More current cytological mappings of the X-chromosome of Drosophila melanogaster have been prepared by a number of investigators. Berendes (1968) found 60% of the bands of Bridges (1938) in Regions 1A through 3F in his electron microscopic study; he later calculated 67% of the bands of Bridges (1938) in the Regions 1A through 4E (Berendes 1970). Sorsa (1969c) also noticed a reduction of band number in an electron microscopic investigation of the salivary gland 3R-chromosome of Drosophila melanogaster. He obtained an average of 90% of the bands shown in the light microscope of Bridges (1942).

From observations of the salivary gland X-chromosome of Drosophila melanogaster in this study from Table V, 49% of the bands of Bridges (1938)

were observed in regions 1A through 10F. There must be some explanation for such a band number reduction when investigated with electron optics. As has been mentioned above, the discrepancy lies in the amount of observable double bands (doublets) in revised chromosome maps of highly stretched chromosomes. With reference to the above mentioned discussion concerning acetic acid, a single band may become vacuolated and appear as a doublet. It is also likely that stretching can transform a single band into a doublet. So then, a single band can either be fixed, squashed or pulled into the form of a doublet, triplet or perhaps any number. It appears in all probability that this stretching was a key factor in the band number increase of the X-chromosomes of Bridges (1938) compared to his earlier map (Bridges 1935).

Although these chromosomes have a high degree of elasticity, the pressure from either thumb or foot squashing and occasional sliding of the coverslip may disturb the fine morphology of such an entity. It is also obvious that the degree of inconsistency in such squashing procedure results in inconsistent data from chromosome to chromosome. Such chromosome mapping, based solely on visual microscopic observations, either with light or electron optics appears then to be a matter of "rather subjective decisions" (Sorsa 1969c).

The steriology of these three dimensional structures must also be discussed. Berendes (1970) and Sorsa (1969c) considered central sections to be the ideal regions for proper band counting. However, it seems highly conceivable from a number of serial sections that a band or granular area can exist in any one plane of section whether it is centrally or peripher-

ally located. Thus, a map based entirely on central sections could very well differ from a map composed from peripheral sections, especially if some of the sections were cut obliquely. Since this study was not based entirely on serial sections, it should be stressed that "mapping based on single sections of the chromosomes may be rather unreliable," according to Sorsa and Sorsa (1969c).

It has been pointed out earlier that only one half of the X-chromosome was investigated. A complete extension of the chromosome from 1A to 20D, ie, from the tip to its attachment at the chromocenter was rare. In the *in situ* condition, the *Drosophila* chromosome arms are wrapped similar to a loosely knit ball of yarn. The addition of cholchicine also did not help straighten out the chromosome arms. This is the reason for the overlapping in some micrographs (Figures 13, 15 and 26).

"Weak points" along the chromosome have been described (Bridges 1935; Arcos-Terán and Beerman 1968). These are regions along the chromosome where the polytenes tend to pull apart such as is seen in Figures 15 and 32 between Regions 10F and 11A. The presence of these weak regions on the chromosome strongly suggest a reason for the failure of observing the entire chromosome.

A genetic representation of the chromosome bands was proposed by Muller and Prokofyeva (1935) who postulated that each band represents one gene. Assuming that this hypothesis is true, it can also be assumed that the band count represents the number of coding units for that particular chromosome. In order to further elucidate this hypothesis, a variety of deficiencies for recombination studies have been studied

(Berendes 1970). In some chromosome regions, a one to one correspondence has been observed between gene and band numbers, in others it has not. As an example, Berendes (1970) observed electron microscopically in the salivary X-chromosome of Drosophila melanogaster 13 bands within the Regions $3A_2$-$3C_3$. These bands correspond to the 13 functional units in this area as described by Kaufmann, Shen and Judd (1969). Judd (1962) located 16 separable loci between the genes zeste and white corresponding to these 13 functional units described above. Table VI shows 13 bands in this same region if we exclude the first doublet band drawn. However, if we include the doublet in $3A_2$, the total increases to 15 bands. (It appears from the map of Berendes (1970) that he does not include $3A_2$ in this summation).

It appears evident in the above region that the number of bands differ only slightly from the known genes (Berendes 1970). Bridges (1938) shows 12 bands (doublets apparently assumed as singlets). Berendes (1970) suggested that the light microscope was not capable of resolving some very thin bands. Rayle and Green (1968), as well, indicated 5 recombinationally separable loci in Regions $3B_2$-$3C_3$. Bridges (1938) shows 4 bands in this region, Berendes (1970) shows 5 although a direct count from his map shows 6 bands if the faintly drawn lines (assumed bands) are counted.

Other areas are not as exact. According to recombination studies of Beerman (1966), Region $3C_2$ through $3C_7$ represents the genes w, rst, vt, fa (spl N). Berendes (1970), however, shows only 3 distinct bands in this region. The "subjectiveness" of band counting thus becomes apparent. These data do, however, indicate that at least for certain

regions a one to one relationship between genes and band numbers appears to exist (Berendes 1970).

The discrepancy in the band numbers as has been presented in the salivary gland X-chromosome of Drosophila melanogaster has significant implications in the calculations of DNA content of the average haploid band. This is due to the fact that counting band numbers has been the sole parameter for such calculations. Rudkin (1965) calculated $3 \times 10^{-5}$ picograms of DNA/band on the basis of 1012 bands counted in the X-chromosome. Berendes (1970) suggested an increase to $4.5 \times 10^{-5}$ picograms of DNA/band in Regions 1A through 4E.

Per cent calculations seen in Table V were based on the data in Tables II, III and IV compared to light microscopic observations of Bridges (1938). Table V also shows comparable data in the present investigation (in most regions) to that of Berendes (1970) in Regions 1A through 4E, ie, some 98% of his bands. This figure is obtained if we include the doublets and the high band count of Region 2B. In Regions 4F through 10F, however, a greater band number reduction was noticed, ie, 41% of the bands of Bridges (1938) was observed. This reduction was thought to be due partially to less observable doublets as compared to the revised map of Bridges (1938). It was also thought that inadequate focusing was a contributing factor to the problem.

It is interesting to note that the doublets, were observed, appeared to follow very closely to the doublets shown by Bridges (1935) in his earlier cytological map. As was pointed out above, certain chromosome regions did not follow the exact band counts as has been observed by

previous investigators. This may be attributable to the manner in which the region bar were drawn, and can be perhaps considered a primary reason for such discrepancy.

From these observations, it is also reasonable to assume both a change (increase) in the average DNA/band in the regions investigated and that the band numbers in the remaining portions of the X-chromosome are proportionally reduced to that of Regions 1A through 10F. These results further indicate continued disagreement with the one band-one gene hypothesis of Muller and Prokofyeva (1935) assuming that these results are reliable.

Additional morphological investigations of the salivary gland X-chromosome of Drosophila melanogaster are in order to further elucidate banding patterns, fine structural morphology and function of the polytene chromosomes. Such efforts may apply to the improvement of a general chromosome model in eucaryotes as well as continuing the search into the realms of molecular genetics. We are presently finding ourselves "out of the woods and upon a clearly marked highway with by-paths stretching in all directions." It is hoped that this highway may someday direct us to the lair of the gene itself (Painter 1934a).

SUMMARY

Results of transmission electron microscopic observations of squashed salivary gland X-chromosomes of Drosophila melanogaster indicate a reduction in band number in Region 1A through 10F compared to the light microscopic, cytological map of Bridges (1938). Although differing only by the method of fixation employed compared to a similar investigation in Regions 1A through 4E by Berendes (1970), 66% of the bands of the cytological map of Bridges (1938) were observed in this area.

A greater reduction of bands were observed from Regions 4F through 10F, ie, 41% and 49% of the bands of Bridges (1938) were observed over the entire chromosome area studied. This overall decrease was thought to be caused primarily by the reduction of observable double bands (doublets) as seen in the electron microscope. This reduction is thought to have significant bearing on the calculations of average DNA concentration per chromosome band. An ultrastructural map representation of the regions investigated is provided.

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