STUDIES ON THE ISOLATION OF METAPHASE CHROMOSOMES

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ABSTRACT

A method for the isolation of metaphase chromosomes from mouse L1210 leukemia cells has been developed. Cells, arrested at metaphase with colchicine, were exposed to hypotonic solution and the pH was then adjusted to 5.6 to stabilize the chromosomes. The metaphase figures were subsequently disrupted and the chromosomes isolated by a series of differential centrifugations in sucrose. The isolated chromosomes were well preserved, as judged by morphological criteria. The effect of various enzymes and chemical agents on the isolated chromosomes was studied. Chymotrypsin, trypsin, and deoxyribonuclease caused a marked disintegration of the chromosomes, whereas treatment with pepsin and ribonuclease induced no significant morphological alterations.

INTRODUCTION

Although the role of chromosomes as the vehicle for the transmission of genetic determinants is well known, the small amount of direct information available concerning their physicochemical and biological properties arises from the difficulties inherent in their isolation. These difficulties are particularly pronounced with chromosomes in interphase since no precise indication of their morphology and structure has appeared. However, the morphological characteristics of chromosomes in metaphase have been detailed in the cells of many species. Accordingly, studies of the isolation of such chromosomes were conducted, and these are the subject of this report.

MATERIALS AND METHODS

Animals and Tumors

DBA_2 or BDF_1 (F_1 of ♀C57Bl × ♂DBA_2) mice were implanted intraperitoneally (IP) with approximately 1×10^6 lymphocytic leukemia L1210 cells. On the fourth day after implantation, the mice were injected (IP) with colchicine (approximately 1 μg/gm body weight) and sacrificed 17 hours later by cervical dislocation. The peritoneal cavity was opened, and ascitic fluid with leukemic cells was removed and pooled in an ice-cooled beaker. The peritoneal cavities were washed with 0.14 M NaCl containing 0.003 M $CaCl_2$. In some experiments heparin (20 units/ml of washing solution) was added to prevent clotting. An average of 2 ml of compact cells was obtained from 25 mice.

The proportion of cells arrested at metaphase ranged usually from 10 to 20 per cent of the total cells, though occasionally the percentage was somewhat greater. When the exposure to colchicine was shortened to 6 hours, the percentage of metaphase cells was lower.

Isolation Procedure

The isolation procedure is illustrated in Fig. 1. All steps were performed in a cold room at 4°.

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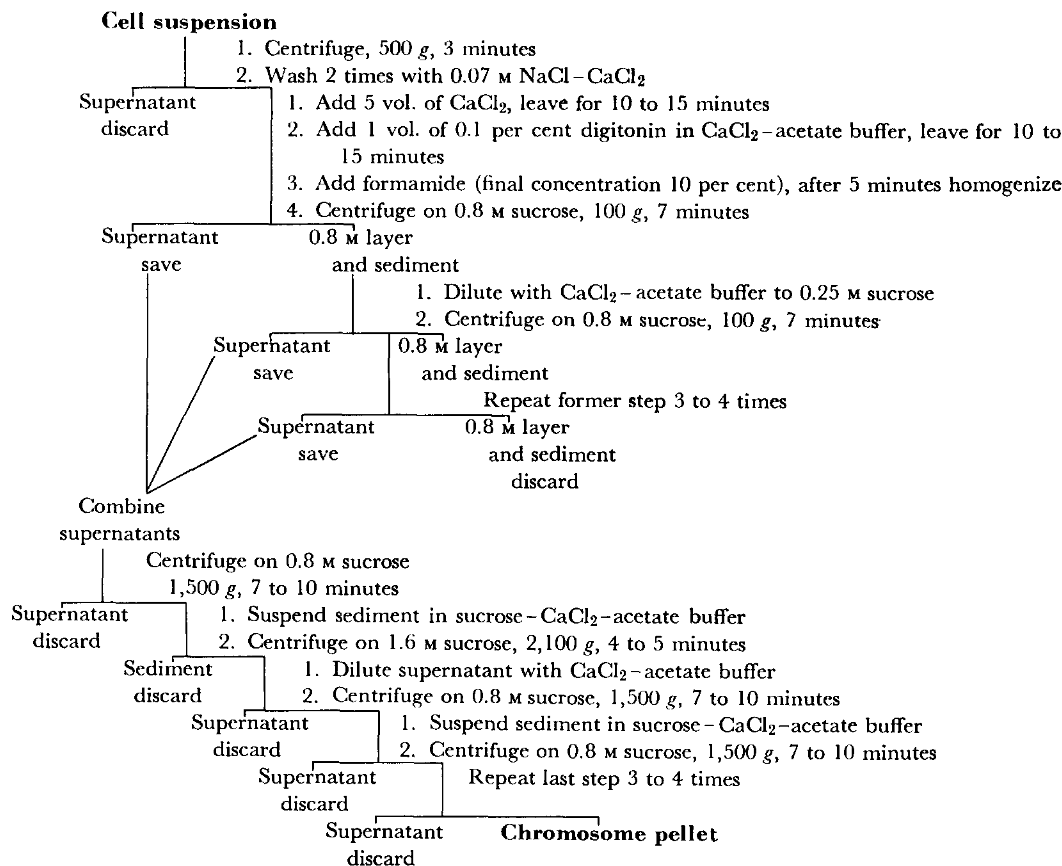

FIGURE 1 Scheme for the isolation of metaphase chromosomes. When not otherwise specified, the concentrations of the solutions were: $CaCl_2$, 0.003 M; $CaCl_2$-acetate buffer, 0.003 M $CaCl_2$ in 0.1 M acetate buffer, pH 5.6; sucrose-$CaCl_2$-acetate, 0.25 M sucrose and 0.003 M $CaCl_2$ in 0.1 M acetate buffer, pH 5.6; 0.8 M sucrose, 0.8 M sucrose and 0.003 M $CaCl_2$ in 0.1 M acetate buffer, pH 5.6.

The cell suspension in 0.14 M NaCl + 0.003 M $CaCl_2$ was filtered through 4 layers of gauze into 50 ml plastic tubes (2.5 × 10 cm) and centrifuged for 3 minutes at 500 *g* in a horizontal head of an International Equipment Co. No. 1 centrifuge. The cells were washed twice with 0.07 M NaCl + 0.03 M $CaCl_2$ in order to lyse erythrocytes, and each time sedimented at 500 *g* for 3 minutes. Thereafter, 5 volumes (in respect to the original volume of compact cells) of 0.003 M $CaCl_2$ in H_2O was added and the suspension allowed to stand for 15 minutes with occasional mixing by a Scientific Industries Co. Vortex Jr.

FIGURE 2 Metaphase figures of mouse L1210 cells after exposure to hypotonic salt solution. Aceto-orcein. × 1,300.

FIGURE 3 Leukemic cells after homogenization. Note intact intermitotic nuclei and dispersed metaphase chromosomes. Aceto-orcein. × 1,300.

FIGURE 4 Sediment after removal of chromosomes by low speed centrifugation. Note intact nuclei and undispersed metaphase figures. Aceto-orcein. × 1,300.

FIGURE 5 Crude chromosome preparation. Note contaminating fine particulate material. Aceto-orcein. × 1,600.

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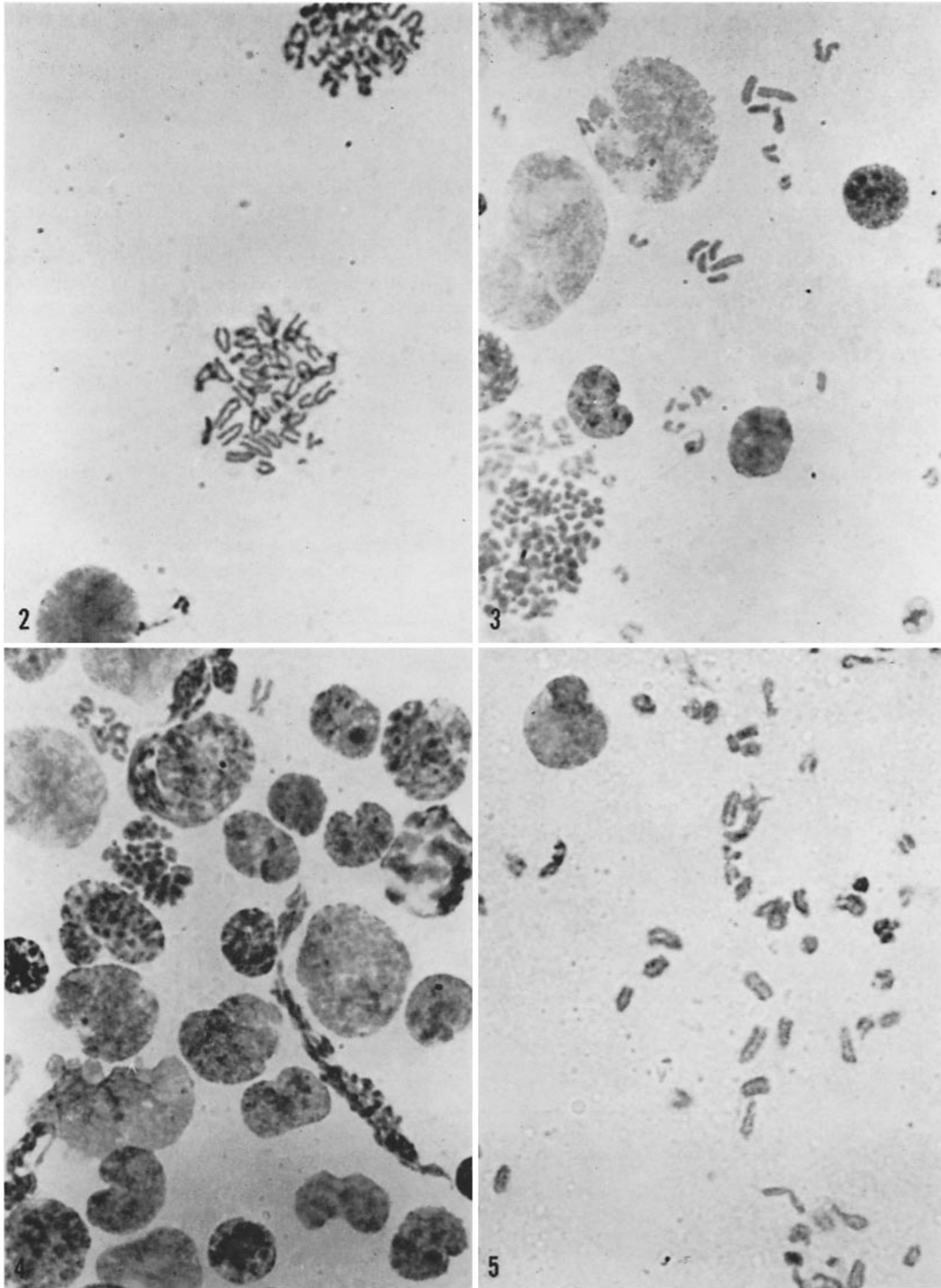

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mixer. Exposure to the hypotonic solution caused a swelling of the cells which in some cases was sufficient to disrupt cell membranes. However, metaphase chromosomes were spread out but retained their structure (Fig. 2).

After exposure of the cells to the hypotonic solution, 1 volume of 0.003 M $CaCl_2$ in 0.1 M acetate buffer, pH 5.6, was added; the suspension was mixed gently, and allowed to stand at room temperature for 10 to 15 minutes. In some experiments more thorough disruption of cell membranes was achieved by use of digitonin. One volume of 0.1 per cent solution of digitonin in 0.1 M acetate buffer (pH 5.6) containing 0.003 M $CaCl_2$ was added to cells suspended in 0.003 M $CaCl_2$, and the suspension was mixed and allowed to stand for 10 to 15 minutes.

Formamide adjusted to pH 5.6 with 1 N HCl was then added to the mixture to a final concentration of 10 per cent. After 5 minutes, about 10 gm of glass beads approximately 3 mm in diameter were added to the tubes which were then sealed with rubber stoppers and shaken vigorously either by hand or mechanically for 2 to 3 minutes. Microscopic examination of the sample stained with aceto-orcein was made to determine the degree of "homogenization."

This kind of "homogenization" was most suitable for leukemic cells, as it led to the disruption of cells in metaphase and dispersion of chromosomes. Whereas intermitotic cells were also disrupted, their nuclei were still intact. At this step the shape and size of chromosomes were well preserved (Fig. 3).

The separation of chromosomes and all other small particles, such as mitochondria and microsomes, from nuclei and intact cells was achieved by low speed centrifugation. The "homogenate" was underlaid with 10 ml of 0.8 M sucrose + 0.003 M $CaCl_2$ in 0.1 M acetate buffer pH 5.6 and centrifuged for 7 to 10 minutes at 100 *g* in a horizontal head. This was sufficient to spin down nuclei, intact cells, and undispersed metaphase figures. It was necessary that the "homogenate" be dilute and no more than 20 ml added to each centrifuge tube. After centrifugation, an upper opalescent layer was easily distinguishable from the 0.8 M sucrose layer. The supernatant layer was pipetted off and saved. Some chromosomes, however, which sedimented at 100 *g*, were recovered from the 0.8 M layer and sediment by dilution with 0.003 M $CaCl_2$ in acetate buffer until the concentration of sucrose was approximately 0.25 M. Formamide (final concentration 10 per cent) was added, the suspension mixed by a Vortex mixer, underlaid with 0.8 M sucrose, and centrifuged again at 100 *g*. The supernatant was saved and the remaining chromosomes recovered from the 0.8 M layer and sediment as described above. This step was repeated 3 to 4 times until microscopic examination revealed only a few chromosomes remaining in the sediment (Fig. 4). The 0.8 M sucrose layer and sediment were then discarded.

The combined supernatants (see Fig. 1) were distributed into 250 ml bottles (40 to 50 ml per bottle) and underlaid with 20 ml of 0.8 M sucrose + 0.003 M $CaCl_2$ in acetate buffer. The chromosomes were sedimented by centrifugation for 15 minutes at 800 to 1,000 *g*. When less than 0.5 ml of packed cells was harvested, 50-ml plastic tubes were used for centrifugation, and the crude chromosome preparation was obtained by sedimentation for 7 minutes at 1,500 *g*. This sediment (Fig. 5) was resuspended in 10 to 20 ml of 0.25 M sucrose (+0.003 M $CaCl_2$ in 0.1 M acetate buffer), underlaid with 0.8 M sucrose, and recentrifuged under the same conditions 3 to 4 times. The sediment was examined microscopically and if chromosomes were contaminated with nuclei and unbroken cells low speed followed by high speed centrifugation (Fig. 1) was repeated.

Better separation of nuclei was achieved in later experiments by the following procedure. A crude chromosome preparation was suspended in 10 ml of 0.25 M sucrose + 0.003 M $CaCl_2$ in acetate buffer. Formamide was added to a final concentration of 10 per cent. The suspension was placed in a 50-ml plastic tube, agitated by a Vortex mixer, and then underlaid with 10 ml of 1.6 M sucrose (sp gr approximately 1.22) dissolved in 0.1 M acetate buffer containing 0.003 M $CaCl_2$ and formamide (final concentration 10 per cent). Both layers were gently mixed with a glass rod until only a 1-cm layer of dense sucrose was left unmixed on the bottom of the tube. The sample was then centrifuged for 4 to 5 minutes at 2,100 *g* in a horizontal head. Thus the nuclei passed through the dense sucrose layer and formed a discrete ring-shaped pellet on the bottom of

FIGURE 6 Isolated metaphase chromosomes; final preparation. Aceto-orcein. × 640.

FIGURE 7 As above. × 800.

FIGURE 8 Isolated metaphase chromosomes. Note well preserved subtelocentric chromosomes. Aceto-orcein. × 1,600.

FIGURE 9 Typical metaphase figure of leukemic L1210 cell. Note subtelocentric chromosome (arrow). Squash preparation courtesy of Dr. J. L. Biedler. Aceto-orcein. × 1,600.

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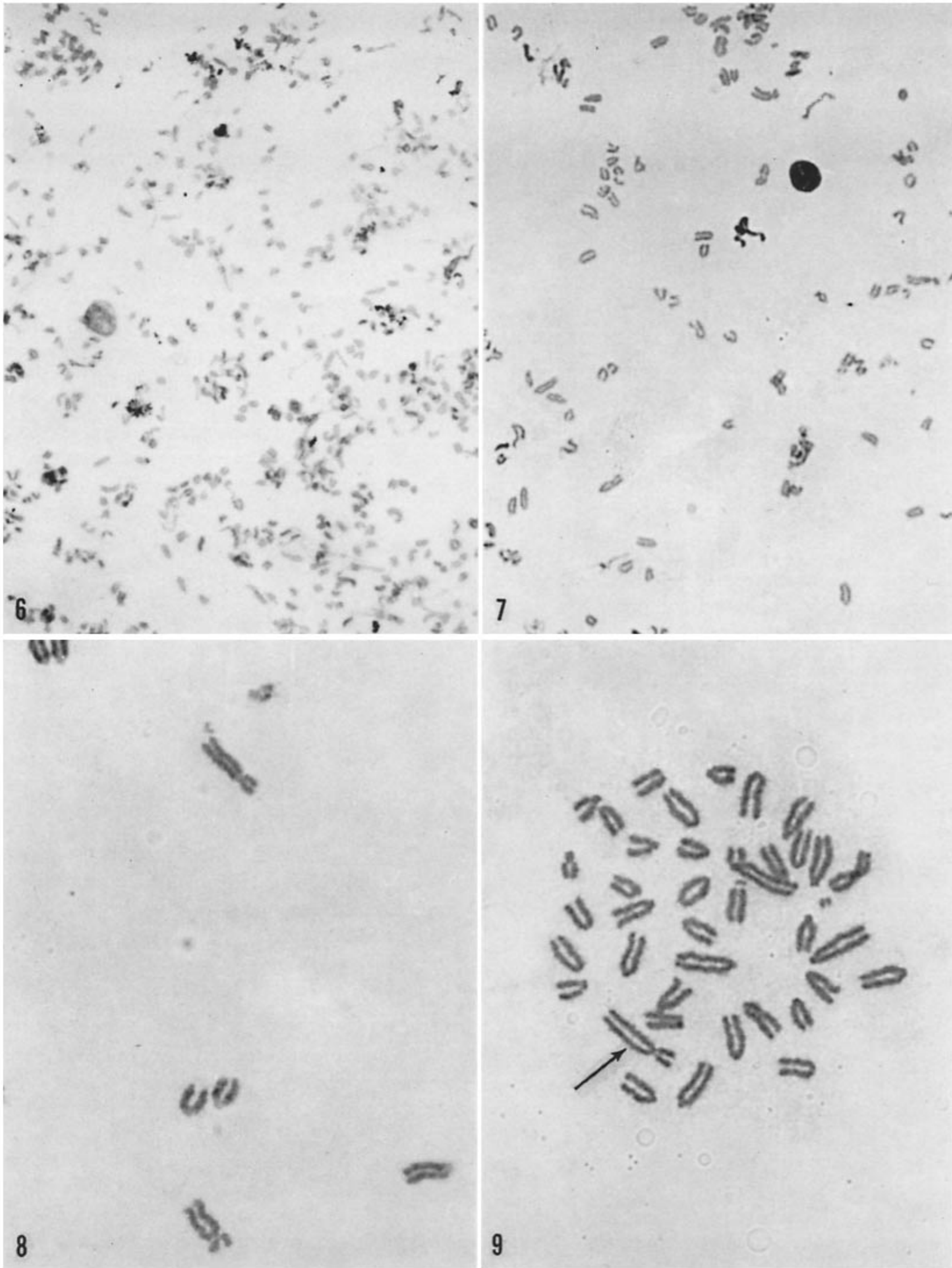

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the tube. The supernatant was pipetted or decanted off and diluted with 0.003 M $CaCl_2$ in acetate buffer until the sucrose concentration was approximately 0.25 M. The chromosomes were sedimented by centrifugation on 0.8 M sucrose for 7 to 10 minutes at 1,500 *g*, and the sediment resuspended in 0.25 M sucrose and recentrifuged (Fig. 1). The final sediment was resuspended in a few drops of 0.25 M sucrose with 0.003 M $CaCl_2$ in 0.1 M acetate buffer. Microscopic examination of this sediment revealed the presence of particles with the typical appearance of metaphase chromosomes (Fig. 6 to 8).

RESULTS

Storage of Isolated Chromosomes

Chromosomes suspended in 0.25 M sucrose + 0.003 M $CaCl_2$ in 0.1 M acetate buffer pH 5.6 were stable morphologically when kept in the frozen state at $-18°$. After 5 months, chromosomes so stored were still well preserved and closely resembled those obtained by the squash technique.

Effect of Different Homogenization Techniques

The proper homogenization of the cells was a prerequisite for the successful preparation of metaphase chromosomes. Several methods of disruption of the leukemic cells were studied, but only the method described above was satisfactory.

Freezing in ethanol–solid CO_2 mixture at about $-70°$ followed by rapid thawing disrupted most of the cells, but some of the nuclei of intermitotic cells were also disrupted. The chromosomes formed either elongated threads or clumps that made further separation impossible. A similar result was obtained with a glass homogenizer (Elvehjem-Potter type), despite attempts to homogenize at various controlled speeds.

The effect of ultrasonic treatment (M. S. E. Ultrasonic Disintegrator, Measuring and Scientific Equipment Ltd., London) on the cells previously exposed to hypotonic shock was extensively studied. It was possible to disperse chromosomes by very short treatment (about 7 seconds at 20,000 cycles/min) but usually a large proportion of nuclei were disrupted, and the final preparation was always contaminated with fragments of nuclei. The length of time of the sonic treatment was critical, since its prolongation for a few more seconds caused disruption of chromosomal structure.

Contamination with nuclear debris resulted when cells were broken by grinding with sand or Al_2O_3 powder. The debris and small particles of Al_2O_3 were impossible to remove by centrifugation.

Forcing of the cell suspension through Whatman No. 1 filter paper in a tissue press caused disruption of the cells and dispersion of metaphase chromosomes. The chromosomes showed a tendency to form threads and total recovery was very poor.

Effect of pH

The importance of pH on the stability of chromosome structure became apparent in a series of experiments carried out at pH values ranging from 5.4 to 7.0. Isolation was usually unsuccessful at pH's 6.4, 6.6, 6.8 and pH 7.0 with 0.25 M sucrose + 0.003 M $CaCl_2$ in 0.05 M tris(hydroxymethyl) aminomethane–maleate (tris–maleate) buffer. At pH 7.0, chromosomes always formed long clumped threads which dissolved readily. As the pH was decreased, the chromosomes became more stable and retained their morphological characteristics. Although isolation at pH 6.4 (maleate buffer) was possible, chromosomes were not well preserved, and nuclear debris was present. At pH 5.6, the chromosome structure was always well preserved, but the disadvantage of the isolation at pH 5.6 was the frequent contamination with aggregates of non-chromosomal material. Microscopically, this material has the appearance of an amorphous mass giving a slightly pink color when stained with aceto-orcein. The amorphous material stained pink-red with methyl green pyronin, suggesting that it consisted of microsomes and ribosomes which formed conglomerates, at a pH below 6.4, that sedimented easily at low speed (11, 12).

Attempts to Remove Impurities from the Chromosome Preparation

To remove contaminating material from chromosome preparations, the effect of sodium deoxycholate, recentrifugation at higher pH, ultracentrifugation in density gradient sucrose, and enzymatic digestion were studied. Sodium deoxycholate at a final concentration of 0.1 per cent mixed with the chromosomes suspended in 0.25 M sucrose in acetate buffer produced an uncoiling effect after 5 to 10 minutes' exposure. This could be prevented somewhat by pretreatment of chromosomes with 5×10^{-4} M 1, 5-pentanedi-

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amine (cadaverine) in 0.1 M acetate buffer, adjusted to pH 5.6. In cadaverine-treated preparations, the chromosomes retained their structure and were resistant to sodium deoxycholate at final concentration of 0.25 per cent for 15 minutes at 4°. However, under conditions of low pH and low temperature (4°) the sodium deoxycholate polymerized to form large aggregates (3) and thus was only slightly effective in disrupting the contaminating materials.

Some degree of purification was achieved by adjusting the final preparation to pH 6.6 and centrifuging the suspension after layering it on dense (sp gr 1.22) sucrose dissolved in tris–maleate buffer pH 6.6 containing 0.003 M $CaCl_2$. The sample was centrifuged for 15 to 20 minutes at 2,100 *g* in a horizontal head. Under these conditions the chromosomes passed through the dense sucrose layer and formed a pellet. Usually some large clumps of amorphous material were still present in the preparation. Resuspension of the pellet and repeated centrifugation allowed further purification. Sometimes the particulate material formed a compact layer on top of the dense sucrose layer and prevented further sedimentation of the particles. Formation of this compact layer could be prevented if the chromosome suspensions were supplemented with formamide (final concentration 10 per cent) and mixed with the upper part of dense sucrose in the centrifuge tube.

To purify chromosome preparations by ultracentrifugation on gradient sucrose solutions, plastic tubes (34 ml) were filled with 5 layers of sucrose of decreasing specific gravity as follows (starting from the bottom): (*a*) 1.33; (*b*) 1.32; (*c*) 1.29; (*d*) 1.26; (*e*) 1.24. On the top layer were placed 3 ml of the chromosome preparation in 0.25 M sucrose + $CaCl_2$ in acetate buffer (pH 5.6). Centrifugation in the SW 25 swinging bucket rotor (Spinco ultracentrifuge model L) at 90,000 *g* (calculated for the bottom of the tube) caused a distribution of bands which was similar for various times of centrifugation. After 90 minutes, the lowest band was located on the top of sucrose of a specific gravity of 1.32. When centrifugation was prolonged to 6 hours, three bands were formed: the lowest was on the top of the 1.32 layer and the upper in the 1.26 layer. Centrifugation for 5 additional hours did not noticeably change the position or the amount of material in the bands. The bands were removed by pipette or by collection of drops from a perforation at the bottom of the tube. The samples were then diluted with $CaCl_2$–acetate solution to approximately 30 ml and centrifuged for 15 minutes at 1,500 *g*. Smears from the sediment were examined microscopically after staining with aceto-orcein or methyl green pyronin.

The lowest band (on the top of 1.33 layer) usually contained the chromosomes, a few nuclei, and small clumps of pyronin-positive material. The chromosomes were well preserved in respect to size and shape. The middle band was of similar appearance. In the upper bands were found amorphous, pyronin-positive material, fat droplets, single cells, and chromosomes.

The most useful method for removing impurities was exposure of a crude chromosome preparation to ribonuclease (RNase) and pepsin. A chromosome suspension (1 ml) in 0.25 M sucrose in acetate buffer pH 5.6 was mixed with 0.1 ml of RNase in acetate buffer containing 50 μg of RNase (2 × recrystallized, Worthington Biochemicals Corporation, Freehold, New Jersey). After the sample was incubated at either room temperature or 37° for 20 minutes, 50 μg of recrystallized pepsin (Worthington) in 0.1 ml of acetate buffer were added and the sample incubated for an additional 20 minutes. The chromosomes were then centrifuged at 200 *g* for 4 to 5 minutes, resuspended in 0.25 M sucrose (+ $CaCl_2$ in acetate buffer) and recentrifuged. The chromosomes treated with both enzymes showed slight changes in shape and size with frequent formation of oval or rounded bodies in which both arms were unrecognizable. However, enzymatic treatment removed almost all RNA-positive material as was revealed by methyl green pyronin staining.

Some Properties of Isolated Chromosomes

In tissue culture media such as 1B7 (27) or 199 (Microbiological Associates, Bethesda) at 37°, chromosomes retained their morphology for at least 6 hours, after which a few of them formed rounded bodies and others were shortened and more compact.

The action of some chemical agents on isolated chromosomes was studied by the drop technique on microscope slides at room temperature. Urea, at a final concentration of 0.5 M, caused a complete uncoiling and dispersion of chromosomes after 15 minutes of exposure. Sodium deoxycholate at 0.02 per cent exerted no harmful effect, but at 0.1 per cent caused disruption of chromosome structure after 5 to 10 minutes.

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In other experiments it was shown that cadaverine and spermine at a final concentration of 5×10^{-4} M protected the chromosomes against the destructive action of sodium deoxycholate at concentrations of 0.1 per cent and 0.25 per cent, but chromosomes preserved with spermine did not survive exposure to 0.5 per cent sodium deoxycholate for 15 minutes. Chromosomes resuspended in distilled water at room temperature retained their structure for 30 minutes.

The effects of chymotrypsin, trypsin, pepsin, RNase, and deoxyribonuclease (DNase) on isolated chromosomes are summarized in Table I. A 0.5 ml solution of one of the above mentioned enzymes (50 μg) was added to 1.0 ml of chromosome suspension in 0.25 M sucrose + 0.003 M $CaCl_2$ in acetate buffer pH 5.6. The samples were incubated at 37° for either 10 or 20 minutes with occasional agitation. The samples were then underlaid with 5 ml of 0.8 M sucrose in acetate buffer and spun for 5 minutes at 1,500 *g*. The sediment was resuspended in a few drops of 0.25 M sucrose in acetate buffer, and smears of this suspension were examined microscopically after staining with aceto-orcein. Chromosomes were readily destroyed by chymotrypsin, trypsin, and DNase, but not by pepsin and RNase when incubated separately or simultaneously, even for prolonged periods of time (Table I).

TABLE I

Effect of Enzymes on Isolated Chromosomes

Enzyme concentration per 1.5 ml sample	Incubation time	
	10 *minutes*	20 *minutes*
50 μg		
Chymotrypsin	chromosomes contracted	chromosomes completely disintegrated
Trypsin	chromosomes completely disintegrated	chromosomes completely disintegrated
Pepsin	chromosomes well preserved	chromosomes well preserved
Ribonuclease	chromosomes well preserved	chromosomes well preserved
Deoxyribonuclease	chromosomes completely disintegrated	chromosomes completely disintegrated
	60 *minutes*	90 *minutes*
100 μg		
Ribonuclease + pepsin	chromosomes contracted	some chromosomes preserved, others formed rounded bodies

Suspensions of chromosomes were exposed to enzymes at 37° for the indicated times. Chromosomes were then sedimented and acetoorcein stained smears were examined microscopically.

DISCUSSION

Mirsky and Ris, pioneering in studies of the isolation and properties of chromosomes (23–26, 29), developed a method which led to the isolation of chromosomal structures from the nuclei of fish and fowl erythrocytes and from the intermitotic nuclei of calf thymus and liver cells. Similar chromatin threads had been obtained previously by Claude and Potter (6) from leukemic cells. Although these structures had the appearance and properties expected of chromosomes, doubt was raised by Lamb (17, 18) and Calvet *et al.* (5) who considered the isolated particles to consist of nucleoprotein threads artificially formed as the result of the isolation procedure. However, support of the procedure of Mirsky and Ris came from studies of Denues (8, 9) who also regarded the threads he obtained from chicken erythrocytes to be isolated chromosomes. Using follicle epithelium of *Drosophila* as a source material, Pfeiffer (28) was able to isolate structures regarded as intermitotic chromosomes. Since morphological

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criteria for the identity of intermitotic chromosomes have not been sufficiently developed (*cf.* reference 10) whereas those of metaphase are well established, cells in metaphase were employed as a source of chromosomes for isolation. There would be, therefore, little ambiguity in the identification of chromosomes isolated from cells in metaphase.

As a source of material, cells of mouse L1210 leukemia in ascitic form were selected because the morphology of their chromosomes in metaphase has been described in detail (1, 2). In particular, these cells show an unmistakable subtelocentric marker chromosome (Fig. 9) which provides an unambiguous morphological criterion for identification and a basis of comparison with the isolated chromosomes (Fig. 8).

Several methods for the disruption of cells in metaphase, release of chromosomes, and preservation of their integrity during subsequent isolation steps were attempted. Drastic procedures of cell disruption, such as applications of a glass homogenizer, freeze-thawing or sonic treatment, also led to disintegration of the chromosomes. Even if the chromosomes retained their morphological integrity under such conditions, considerable contamination with cell debris was always present in the preparation. The exposure of the cells to highly hypotonic solutions as employed in cytology (13) was a helpful step since it allowed the dispersion of metaphase chromosomes, swelling of the cells, and in many instances cell lysis. To disrupt metaphase figures and disperse chromosomes the latter must be stabilized.

The best stabilization of chromosome structure was achieved by lowering the pH of the cell suspension below 6.0. The maintenance of chromosome structure at low pH probably occurs through stabilization of proteins, especially of histones, which are part of the chromosome architecture. The nucleohistones are transformed into a gel at neutral and alkaline pH, and the chromosome architecture can no longer be preserved. The stabilizing effect of low pH (pH 5.0) on *Sciara* salivary gland chromosomes was pointed out previously by Mazia (20), and also used effectively in the isolation of the sea urchin mitotic apparatus by Kane (14).

The presence of calcium ions in the isolation media described here also prevents chromosome clumping and inhibits their uncoiling and thread formation. Calcium ions are required for the maintenance of the chromosome structure, as has been observed in the isolation of giant chromosomes of *Chironomus* larvae salivary glands (15). Chromosomes from the salivary gland of *Drosophila melanogaster* can be disrupted easily when calcium and magnesium ions are removed by chelating agents (21). Steffensen (31) demonstrated clearly that a more frequent chromosome fragmentation occurred in *Tradescantia* when it was grown on calcium-deficient media. Magnesium ion deficiency caused similar aberrations (30), but calcium ions cannot be efficiently substituted by magnesium. Calcium also exerts a stabilizing effect on the sea urchin mitotic apparatus (22).

The conditions of a low pH and the presence of calcium ions must be maintained throughout the isolation procedure. Isotonic or hypertonic sucrose proved to be useful as an effective stabilizer which served also as a medium for chromosome storage.

The main drawback in use of a pH below 6.0 was the formation of aggregates of materials of high RNA content which were difficult to remove from the final preparation. These aggregates are probably composed of microsomes and ribosomes which clump at lower pH values and sediment easily at low centrifugal forces (11, 12). Besides a low pH, sucrose is probably another factor responsible for the coalescing of microsomal particles isolated from *Azotobacter vinelandii*. Prevention of the formation of these conglomerates proved difficult since the main factor responsible for their formation, *i.e.* low pH, cannot be changed without destroying the chromosomes. Since formamide possesses a high dielectric constant, its possible dispersing effect was studied and found to be effective; at 10 per cent final concentration formamide did not affect the chromosome structure.

The removal of extrachromosomal RNA from these preparations proved to be the most difficult problem of the isolation procedure. Ultrasonic treatment and sodium deoxycholate are widely used for disruption of microsomal membranes and dispersion of ribosomes, but these proved destructive to the chromosome structure. The destructive effect of deoxycholate could be inhibited to some extent by pretreatment of chromosomes with cadaverine and spermine. Diamines, polyamines, and basic proteins were reported to exert a protective effect against heat denaturation of DNA of calf thymus and *Bacillus subtilis* (33), to stabilize transforming DNA of *B. subtilis* (32), and also to inhibit the uncoiling process of

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chromosomes at anaphase (7). Perhaps a sufficient protection of chromosomes could be achieved with other polyamines and basic proteins at neutral pH so that simultaneous treatment with mild detergents to prevent formation of contaminating microsomal aggregates could be effected. This approach will be examined in future investigations.

The most useful procedure for removal of extrachromosomal RNA-positive material from chromosome preparation was treatment with RNase and pepsin. The structure of chromosomes in the preparations was readily disrupted by exposure to chymotrypsin, trypsin, and deoxyribonuclease. A similar action of trypsin on the structure of *Sciara* chromosomes has originally been described by Caspersson (quoted in reference 20) and confirmed by Mazia (19, 20). On the other hand, pepsin and papain did not destroy *Sciara* salivary chromosomes (19, 20), although pepsin caused shrinkage. Similar observations were noted with our preparations (Table I). Although deoxyribonuclease causes disintegration of lampbrush chromosomes as well as the isolated mouse chromosomes, there are differences in the action of other enzymes; the lateral loop structures (but not the linear structures) of lampbrush chromosomes are dissolved by ribonuclease and by pepsin (4). These enzymes do not induce a dissolution of the mouse chromosomes.

In order to remove extrachromosomal RNA-positive material from the chromosome preparations, both RNase and pepsin were employed. RNase alone was not sufficient. This is reminiscent of the finding by Ts'o (34) that microsomal protein remaining after RNase treatment also aggregates and is insoluble at the pH range 4 to 11. However, the usefulness of this method of purification is limited, since it is known that ribonucleic acid and non-histone proteins are constituents of chromosomes which can be removed by enzymatic digestion (16).

The analogous procedure as used for L1210 ascites cells was applied to L1210 solid tumors, sarcoma 180 growing in ascitic form, and Ehrlich ascites carcinoma cells. In none of these cell lines was isolation successful. The isolation of the chromosomes from sources other than L1210 ascitic leukemic cells may be possible, provided proper methods for disrupting the cells are developed. However, the growth rate of L1210 cells is greater than that of other cell lines, and consequently we might expect a higher percentage of L1210 cells to be arrested at metaphase by colchicine.

Although the morphological and staining characteristics of the isolated chromosomes leave little doubt as to their identity as metaphase chromosomes, their state of purity does not yet permit an instructive chemical analysis of their composition. The chemical analyses thus far carried out show an inordinately high concentration of RNA, but this is probably due to contaminating cytoplasmic RNA particles, removal of which has not yet proved feasible. However, the chromosome preparations at their current level of purity appear to be adequate for studies on their penetration into mammalian cells. Such experiments have been carried out and are presented in the following manuscript.

Dr. Chorąży participated in this study while he was a Visting Research Fellow of the Rockefeller Foundation.

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