Table I. Axial growth of normal collagen fibrils at 30 °C in phosphate buffer pH 7.0. I = 0.2, $C_o$ = 750 μg/ml.

| Specific activity of labelled collagen | N end (molecules/sec) | C end |
|---|---|---|
| 0.4 mCi/mg | 1.73 ± 0.06 | 1.27 ± 0.08 |
| 0.2 mCi/mg | 1.89 ± 0.08 | 1.11 ± 0.06 |

Errors represent standard deviations on measurements of about 20 fibrils in each case.

Table II. The energies of $\beta^-$'s emitted by $^{125}I$ (taken from reference[4])

| Energy (keV): | 2.77 | 3.6 | 22.5 | 31.0 | 34.3 |
|---|---|---|---|---|---|
| Fraction of $\beta^-$'s with this energy | 0.291 | 0.488 | 0.142 | 0.067 | 0.012 |

The high proportion of electrons with low discrete energies is due to their atomic, rather than nuclear, origin. They are emitted in consequence of the electron capture and $\gamma$-emission processes by which the $^{125}I$ nucleus decays and are a mixture of internal conversion and Auger electrons[14].

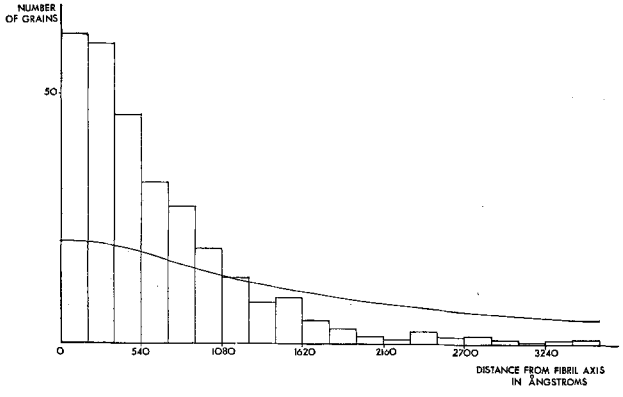


Fig. 2. For explanation see text.

of 540 A was obtained from 600 grains, after a correction for this background had been made.

The theoretical curve in Figure 2 represents the probability of a hit as a function of distance, assuming that electrons pursue a straight line path and are not absorbed. It is derived from the curve of DONIACH and PELC[11] for a point source, by numerical integration over a line source of fibrillar dimensions in the same geometry as the experiment. The large difference between the histogram and the curve (whose areas are the same) indicates that the assumption about electron paths is not valid for $^{125}I$. This is likely on theoretical grounds. Since most of the decay electrons of $^{125}I$ have a very low energy (see Table II), these will have a range in silver bromide of less than a single L4 crystal diameter[12]. Shielding of one crystal by another will therefore be significant, resulting in an enhanced probability of hits directly over the source and an improvement of resolution[13].

Our results confirm the potential of $^{125}I$ for electron microscope autoradiography and demonstrate the high resolution that can be achieved. They show that problems arizing from the effects of iodination on protein behaviour can be minimized by a suitable method of iodination.

*Résumé.* Le $^{125}I$ possède un grand potentiel pour l'autoradiographie en microscope électronique. Il permet d'atteindre la haute résolution. Le nombre des problèmes que posent les effets de l'iodination sur le comportement des protéines peut être considérablement réduit par une méthode appropriée d'iodination.

R. A. HAWORTH[15,16] and J. A. CHAPMAN[17]

*Department of Medical Biophysics, The University, Manchester, M13 9PT (England), 18 June 1974.*

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[15] Present address: Institute for Enzyme Research, Madison, Wisconsin 53706 USA.
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[17] To whom requests for reprints should be sent.

## Differential Staining of the Satellite Regions of Human Acrocentric Chromosomes

Cytogeneticists have reported that all 10 acrocentric chromosomes of the D and G groups in humans probably bear satellites on their short arms; furthermore, all of these could be expected to function in the organization of nucleoli[1,2]. Certain of these acrocentric chromosomes approach each other with their satellite ends more often than would be randomly expected[3,4]. Cytogeneticists are presently investigating this preferential satellite association as a possible cause of chromosomal non-disjunctions and translocations[5]. However, these investigators are handicapped because these regions do not usually stain and they have to arbitrarily define these associations. OHNO et al.[2] suggested that at metaphase the satellite regions become very short and stain poorly because of the deficiency of DNA. Therefore, there was a need for a technique to differentially stain these important satellite regions.

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It was recently found that the satellite regions of all 10 D and G group chromosomes could be differentially stained with Giemsa after extraction of both nucleic acids and histone proteins[6]. Using autoradiographic evidence, HENDERSON et al.[7] have shown that actual physical connections exist between many of the associating acrocentric chromosomes. In our laboratory, we have developed a much easier technique which differentially stains the human acrocentric satellite regions as well as the physical connectives of satellite associations, by using a modification of an ammoniacal-silver procedure[8–12].

*Materials and methods.* This procedure, which we call the AS-SAT technique, requires the use of 4 solutions for the differential staining of the satellite regions. 1. A pretreatment solution of 10% formalin which is neutralized with 3.2 g of sodium acetate crystals ($CH_3COONa \cdot 3H_2O$). 2. A prestaining ammoniacal-silver solution (A–S I) which is prepared by dissolving 5 g of silver nitrate into 50 ml of distilled water. Immediately 40 ml of this aqueous silver nitrate solution are added to 5 ml of concentrated ammonium hydroxide in a slow manner with constant stirring. Then the remaining 10 ml of the aqueous 10% silver nitrate are added dropwise to this solution until it reaches its first permanent faint turbidity. The A–S I solution has a pH of 10–11. 3. A staining solution (A–S II) which is prepared by adding 8 g of silver nitrate to 10 ml of acetate buffer, pH 3.6 (stock buffer prepared by adding 46.3 ml of 0.2 *M* acetic acid and 3.7 ml of 0.2 *M* sodium acetate). Upon the dissolving of the silver nitrate crystals, the entire solution is added to 15 ml of concentrated ammonium hydroxide. This solution is colorless and has a pH of about 12. 4. A developing solution of 3% formalin, neutralized with sodium acetate crystals.

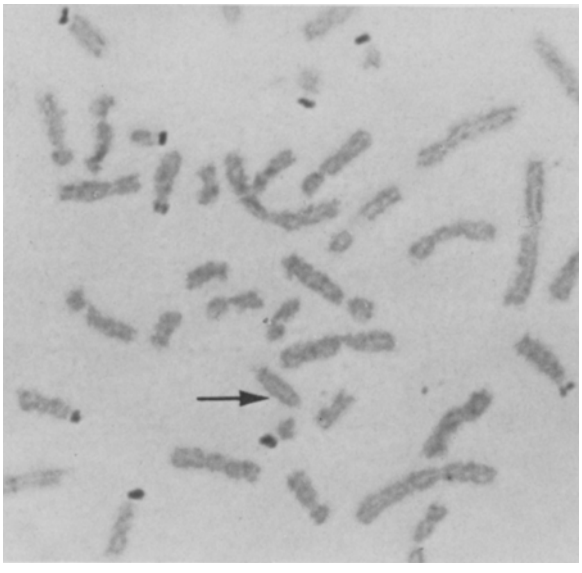

Fig. 1. Metaphase chromosome spread (from abnormal child) exhibiting 9 differentially stained satellites which appear as dark areas above the kinetochore of the acrocentric chromosomes. Arrow points to D group chromosome which lacks a satellite staining region.

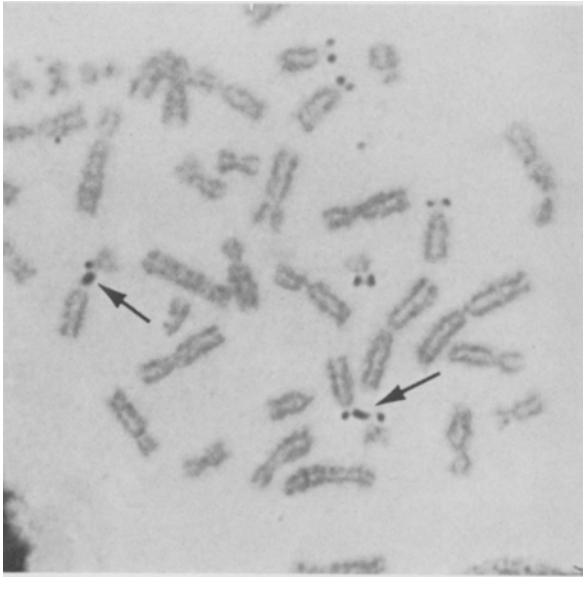

Fig. 2. Metaphase chromosome spread (from abnormal child) exhibiting only 8 differentially stained satellites of which 4 are involved in D–G satellite associations. The physical connections between the D and G group associations are indicated by the arrow.

Microscope slides containing air-dried human chromosomes from leukocyte culture[13] are immersed into a coplin jar containing 10% neutral formalin. After 15 min, the slide is removed and rinsed quickly in 3 changings of deionized water and placed into the A–S I prestaining solution for 15 sec. It is then removed, rinsed, and placed into a 0.07 *N* NaOH solution for 5 min. It is then rinsed thoroughly for 1 min in deionized water and blotted dry. Equal amounts (approximately 3–4 drops) of first 3% formalin and then A–S II solution are pipetted onto the slide surface and covered with a coverglass. The progress of the staining reaction can be followed under the microscope, first using phase-contrast microscopy, followed by bright field illumination as the staining intensity gradually increases. The chromosomes are usually sufficiently stained within 5 min. If the chromosomes are understained after 5 min, the coverglass may be rinsed off under running tap water, the slide blotted dry, and restained with A–S II and 3% formalin. After the proper differential staining is reached, the coverglass is rinsed off and the slide is soaked for 5 min in xylene, and permanently mounted in a suitable mounting medium. The attachment of the silver stain to the satellite regions greatly darkens these areas and they are most conspicuous when the remainder of the chromosomes and chromosome arms are light yellow to orange in color. Overstaining causes a general darkening of the chromosomes and the differential staining is lost.

*Results and discussion.* Figure 1 illustrates a metaphase chromosome complement stained by using the AS–SAT technique. These metaphase chromosomes were obtained from an abnormal newborn, which died within 3 days after birth. Of 50 metaphase plates examined from this infant, 9 satellited chromosomes were evident in 41 cells, 8 satellited chromosomes in 7 cells, and 7 satellited chromosomes in 3 cells. No cell examined had all 10 acrocentric satellite regions stained and the unstained

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acrocentric, which was detectable in most cells, was of the D group. Figure 2 shows 8 satellited chromosomes from the same infant. Two acrocentric D-G satellite associations can be seen clearly. 35 of the 50 metaphases examined contained such acrocentric associations. 21 of these were D–G associations, 9 were D-D, and 5 were G-G.

In apparently normal individuals, the number of acrocentric chromosomes which exhibited differentially stained satellites varied from person to person but each individual tended to have a specific number. For example, a normal 8-year-old male consistently had 10 satellited acrocentrics as did his 10-year-old sister. However, a normal 22-year-old male had only 5 such satellited chromosomes. Whether this variation is real or represents variability in the technique is not presently known. However, experiments with control slides indicate that the variation is real.

The question arises as to the precise cytochemical composition of the satellite staining regions. Past investigators have suggested that these satellite regions are nucleolar organizers[1,2]. HENDERSON et al.[7], using autoradiographic evidence, indicated that the acrocentric satellited regions might be ribosomal DNA. COMINGS et al.[14] suggested that these regions contain ribosomal RNA (which may or may not be associated with heterochromatin). MATSUI and SASAKI[6] disagreed with these nucleic acid hypotheses because their own cytochemical studies indicated that these satellites are composed of acidic proteins. We, too, have obtained evidence that the acrocentric satellite regions are composed of the acid-insoluble or acid proteins. Treatment of the chromosomes with both DNase and RNase (Sigma: 100 $\mu$g ml$^{-1}$ at 37 °C for 60 min) did not eliminate the satellite staining regions. When the proteolytic enzyme, Pronase, was used (Cal. Biochem.: 100 $\mu$g ml$^{-1}$ at 37 °C for 10 min) the satellite regions were destroyed. To determine the nature of these proteinaceous staining regions, both acid and alkaline pretreatments of chromosomes were employed. Acid treatment, using 0.2 *N* $H_2SO_4$ for 1-, 10-, and 20-min intervals, left the satellite regions intact. Alkaline incubation in 0.1 *N* NaOH at room temperature for 1 and 5 min did not visibly affect these satellite regions whereas longer treatments for 10 and 20 min eliminated them completely in most cells. In some cells 2 satellite regions still remained intact and these were consistently located on acrocentrics of the D group.

Because of their insolubility in acid and slow solubility in dilute alkali, the satellite staining regions seem to possess a protein fraction which is essentially acidic in nature. However, caution must be taken against rigidly interpreting these regions as being exclusively acid protein because BENJAMIN et al.[15] described a chromosomal RNA which was bound to histone, and was ribonuclease insensitive.

The role of acid proteins in the nucleus is still obscure, even though it has been shown that these proteins quantitatively represent the major constituent of the nucleus[16]. Several authors have stated that some of these proteins may be parts of ribosomes[17–20], or contain RNA[21–24]. More recently, data have been obtained which suggest that the nonhistone chromosomal (or acid) proteins may be responsible for the regulation of gene expression[25], particularly the control of transcription in the cell cycle[26,27].

The acid proteins may play an important role in the size and organization of the nucleolus. Researchers have found that the nucleolus contains both acid-soluble and acid-insoluble proteins, which range from 40–90% of the nucleolar mass[28–32]. BUSCH et al.[33] found the nucleolar acid-insoluble proteins to be present in significantly greater concentrations than the acid-soluble ones. It has also been noted that the nucleoli of cancer patients are larger than those of normal adults[34–36]. Perhaps there may be some relationship between the number and size of acrocentric satellite staining regions and the size of the nucleolus. If so, acrocentric satellite staining regions may be of much importance, especially in comparative studies of the relative staining properties of these regions in normal and abnormal patients. However, these hypotheses require a great deal of future research[37].

*Zusammenfassung.* Es wird eine neue Ammoniak-Silber-Methode für das Färben menschlicher Chromosomen in der Metaphase beschrieben, wodurch die Satelliten aller 10 akrozentrischen Chromosomen zu unterscheiden sind.

W. M. HOWELL[38], T. E. DENTON and J. R. DIAMOND[39]

*Department of Biology, Samford University, Birmingham (Alabama 35209, USA); and Section of Ecology and Systematics, Langmuir Laboratory, Cornell University, Ithaca (New York 14850, USA),*
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[38] The bulk of this research was done while a member of The Section of Ecology and Systematics, Cornell University, Ithaca, New York.
[39] Section of Ecology and Systematics, Langmuir Laboratory, Cornell University, Ithaca, New York 14850, USA.