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by Light Microscopy, Quinacrine Fluoromicroscopy and Autoradiography

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Part of the Y chromosome in humans is detectable as a brightly fluorescing body in interphase nuclei stained with quinacrine dyes. This fluorescent body when stained with quinacrine mustard dihydrochloride, in cells from some 15 human subjects, was seen to manifest a variable morphology which appeared to be related to the stage of the nucleus in the cell cycle. The variable morphology was seen particularly in cultured fibroblasts and in peripheral blood lymphocytes undergoing blastic transformation induced by PHA, but was not observed in buccal mucosa or hair root sheath cells.

The morphology of the Y-body ranged from a highly condensed configuration in small non-transformed nuclei to increased degrees of dispersion which appeared to show direct relationship to size of the transformed nucleus. In nuclei which appeared to be in preprophase (G-2), the Y-body appeared to partially recondense.

Lymphocyte cultures, which were serially harvested, showed an increase in percent of nuclei with a dispersed Y-body, proportional to time in culture, and generally an increase in nuclear diameter.

Nuclei studied from serially cultured skin fibroblasts showed
the highest percent of nuclei with a dispersed Y-body during the log phase of cell growth, and a decline in the frequency of dispersed morphology in the post-log phase. Percent dispersion also showed a direct relationship to mitotic index.

Continuous terminal and pulse-labeling studies of the Y-body revealed increased incorporation of tritiated thymidine with increased dispersion. The labeling studies suggested that the observed morphological configurations were sequential, and that the Y-body while condensed generally lagged in DNA-synthesis, but that the rate of DNA-synthesis of the Y-body was more rapid than the rest of the nucleus as it became more dispersed.

The studies indicate that the different morphologies of the Y-body represent stages in the cell cycle, and suggest that portions of the Y-body may be uncoiling during the time it is actively synthesizing DNA.
Study of the Human Y Chromosome in the Interphase Nucleus by Light Microscopy, Quinacrine Fluoromicroscopy and Autoradiography

by

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Fluorescent Staining of the Human Y Chromosome

The use of quinacrine fluorescent dyes to differentially stain human chromosomes, pioneered by Caspersson et al. (1970a) and Caspersson, Zech and Johansson (1971a), has made it possible to identify each chromosome by its characteristic fluorescent banding patterns. The most intense fluorescence occurs on the distal end of the long arm of the human Y chromosome as reported by Zech (1969) using quinacrine dihydrochloride mustard (QM), and by George (1970) and Vosa (1970) using quinacrine dihydrochloride.

The synonymity of a bright fluorescent body in interphase nuclei stained with quinacrine dyes and the Y chromosome was demonstrated by Pearson, Bobrow and Vosa (1970). A brightly fluorescing body was observed in the nuclei of buccal mucosal epithelium and in cultured skin fibroblasts from normal (XY) males while two such bodies appeared in nuclei of XYY males. These structures were given the name "Y-bodies".

Since these initial observations, quinacrine staining has been used to detect the Y chromosome in a variety of human tissues. The "Y-body" has been demonstrated in hair root sheath cells (Cervenka, Jacobson and Gorlin, 1971; Schwinger et al., 1971), in cells from amniotic fluid (Rook et al., 1971; Mukherjee, Blattner and Nitowsky,
1971), in liver cells (Linder, personal communication), in polymorphonuclear leukocytes (Lamborot-Manzur, Tishler and Atkins, 1971), and in Wharton's jelly from the umbilical cord of newborns (Greensher et al., 1971). Quinacrine staining has been used to detect human sperm bearing the Y-chromosome (Kelberman, Barlow and Vosa, 1970) and to distinguish between X, Y, and YY chromosome containing sperm (Sumner, Robinson and Evans, 1971). The Y fluorescence is detectable in meiotic cells of human males (Pearson and Bobrow, 1970a), a fact which has been utilized to show that the short arm of the Y chromosome is associated with the X chromosome during meiotic pairing (Pearson and Bobrow, 1970b). The brightly fluorescing end of the Y chromosome has so far been found only in man and in the gorilla. Attempts to find a similarly fluorescent region on the Y chromosome of other primates and mammals have been unsuccessful (Pearson et al., 1971).

**Frequency, Size and Morphology of the Fluorescent Y-body in Interphase**

Existing evidence indicates that the fluorescent Y-body in interphase represents the material present on the distal end of the Y chromosome in metaphase. The fluorescent banding patterns of human chromosomes appear to be highly consistent (Caspersson, Lomakka and Zech, 1971b). The intensity of individual bands appears to be unaltered by chromosomal rearrangements (Caspersson, Lindsten and Zech, 1970c; Caspersson, Modest and Zech, 1970d; Caspersson et al., 1971c; Wyandt et al., 1971; Reiss et al., 1971). In an individual
with an isochromosome for the long arm of the Y chromosome, fluorescence of the distal ends of both arms was observed and examination of interphasic nuclei revealed a corresponding dual structure in the majority of Y-bodies encountered (Borgaonkar and Hollander, 1971). In the same study, apparently normal individuals lacking a fluorescent segment of the Y chromosome showed a corresponding absence of Y-body fluorescence in interphase nuclei. It has been noted that the size of the Y-body at interphase varies with the amount of fluorescent material present on the Y chromosome in metaphase (Lewin and Conen, 1971; Cagné and Laberge, 1971).

Some morphological variation has been noted for the Y-body in interphase nuclei from individuals with a morphologically normal Y chromosome. Pearson et al. (1970) noted the presence of the Y-body in 25-50 percent of nuclei of normal XY males examined. Ten to twenty percent of these cells had a Y-body with an apparent double structure "...with the two halves appearing quite widely separated... but appearing smaller than the more common single structure." The frequency of the Y-body in interphase has been reported for different tissues. Cervenka et al. (1971) have noted frequencies ranging from 70-75 percent in hair root sheath cells from normal males, whereas Schwinger et al. (1971) reported a frequency range of 15-55 percent. Similar discrepancies in frequency of the Y-body have been reported for cells from amniotic fluid: 3-9 percent (Rook et al., 1971) versus 40-87 percent (Cervenka et al., 1971). In cultured fibroblasts the frequency of the Y-body was shown to range from 6.8 to
100 percent depending on the state of cell growth (Therkelson and Peterson, 1971). Frequencies in spermatozoa from normal males have been more consistent: (38.5-46.5 percent) reported by Kelberman et al. (1970); (35.5-47.0 percent) reported by Sumner et al. (1971).

In individuals with more than one Y chromosome, the frequency of cells with two Y-bodies is somewhat lower than the frequency of cells showing but one Y-body. A small percentage (1-2 percent) of cells showing more than one Y-body has been reported for normal individuals (Pearson et al., 1970), and 0.9-1.26 percent of spermatozoa from normal males reportedly show two Y-bodies (Kelberman et al., 1970; Sumner et al., 1971).

The X-Chromatin Body and the Y-body

In mammalian female cells, Barr and Bertram (1949) often observed a chromatin body. This body has been termed the "sex chromatin body". However in light of the recent discovery of the Y-body or "Y-chromatin" the term "X-chromatin" seems more appropriate when referring to female sex chromatin, and henceforth will be referred to as such in the remainder of the text.

Much of the data relating to the "X-chromatin body" have been reviewed by Moore (1966). Irrespective of whether a Y chromosome is present or not in a cell, the general rule has emerged that the number of X-chromatin bodies equals the number of X chromosomes minus one.

Two key facts concerning the Y-body are of interest here.
First, the number of Y-bodies in a cell is equal to the number of Y chromosomes present in the cell. Second, whereas the X-chromatin body is presumed to represent an entire X chromosome, the Y-body represents only the brilliantly fluorescent distal portion of the Y long arm.

Widespread attention has been given to the behavior of the X chromosome in female mammals. It has been described as the heterochromatic, heterocyclic, allocyclic or heteropycnotic X, all of which imply a behavior that is unique. The late replicating nature of the heterochromatic X has become well established (Priest, 1968; Miller, 1970), and it has been shown to be genetically inactive at those loci which have been tested (Lyons, 1968).

The Y chromosome in mammals has also been shown to be somewhat heterocyclic and late labeling (Schmid, 1963; Kikuchi and Sandberg, 1965; Miller, 1970), though not as late as the female X. The Y has been generally regarded as the second latest labeling chromosome. It has also been shown by non-fluorescent techniques that the Y chromosome may be partially or completely heterochromatic (Sasaki and Makino, 1963; Arrighi and Hsu, 1971). The heterochromatin of the Y and other chromosomes, however, has been distinguished by the term "constituitive heterochromatin" as opposed to "facultative heterochromatin" of the X (Brown, 1966; Arrighi and Hsu, 1971). To date, no discrete genes have been linked to the Y chromosome.

The recently discovered Y-body can be compared to the X-chromatin body of females. The frequencies that have been reported
for the Y-body are reminiscent of the frequencies that have been reported for the Barr body. Attempts to explain why the X-chromatin body only appears in less than 100 percent of female nuclei, however, have not been entirely satisfactory (Klinger et al., 1967; Comings, 1967b; Mittwich, 1967a and b). Evidence that the X-chromatin body does not uncoil during DNA synthesis has been particularly convincing (Comings, 1967a).

A difference between the Y-body and the X-chromatin body is suggested by the mechanism of QM staining itself. The X chromosome does not fluoresce as intensely as the distal end of the Y chromosome and generally does not appear as a fluorescent body in the interphase nucleus of females. The currently proposed mechanism of QM staining is that QM is intercalating into G-C pairs in the DNA molecule (Caspersson, Zech and Johansson, 1970b). Such regions are postulated as being highly redundant. If this hypothesis is correct, the fluorescing region of the Y chromosome in man and in the gorilla may represent an evolutionary mechanism by which genetic material determining sex in higher mammals is less vulnerable to the effects of mutation. Since the female has two X chromosomes, a similarly effective mechanism may operate by "turning off" abnormal X chromosomes, should they occur (Miller, 1970). Variation in the length of the fluorescence segment on the end of the Y chromosome appears to have little effect on the phenotype of the individual (Borgaonkor and Hollander, 1971; Schnedl, 1971), whereas variations in the amount of the proximal portion of the Y chromosome and the short arm seem...
to have a pronounced effect (Angell, Giannelli and Polani, 1970; Fraccaro et al., 1971; Bühler et al., 1971).

**Purpose of the Study**

Fluorescent staining of the human Y chromosome provides the only opportunity for following the behavior of a single chromosome through the entire cell cycle. In a preliminary examination, by the author, of interphase nuclei of lymphocytes using QM staining it became evident that the Y body had a variable morphology which appeared to be related to the stage of the nucleus in the cell cycle. A similar observation has since been reported by Gagné and Laberge (1971). The behavior of the X and the Y now needs to be compared and contrasted.

The purpose of the present study was to look in considerable detail at the behavior of the Y chromosome during interphase, at its DNA synthetic activity, and at factors affecting the frequency of the Y-body in the interphase nucleus.
II. MATERIALS AND METHODS

Source

Fifteen human male subjects, whose karyotypes were determined from metaphase spreads of cultured peripheral blood lymphocytes, provided materials for the study. Nine of the subjects who were studied most extensively will be identified according to karyotype (number of chromosomes/sex chromosomes) as follows:

Individuals I-IV: 46/XY, normal males with average-sized Y chromosomes but with the length of the fluorescent segment ranging from one-third to one-half the length of the long arm.

Individual V: 46/XY, normal male with an average-sized Y chromosome but with only a short fluorescent segment on the end of the long arm.

Individual VI: 46/XYq+, male with a very large Y chromosome of which approximately three-fourths of the end of the long arm was highly fluorescent.

Individual VII: 47/XXY, Klinefelter male with a normal-sized Y and approximately one-third the length of the long arm being highly fluorescent.

Individual VIII: 47/XYY, male having two average-sized Y chromosomes of identical morphology and approximately one-half the length of the long arm being highly fluorescent.

Individual IX: 48/XXYY, Klinefelter male having two average-sized Y chromosomes of identical morphology but approximately one-third the length of the long arm being highly fluorescent.
The remaining subjects studied less extensively included an additional XXYY male, an XYY male, an XYYY male, and three additional XY normal males.

Interphase nuclei of peripheral blood lymphocytes were studied from all of the subjects. In addition, cultured skin fibroblasts, buccal mucosa cells, and hair root sheath cells were obtained from Individuals I and VI-IX. In the cases of the additional XYY, XXYY and XYYY males, 72 hr lymphocyte cultures were examined from slides obtained from other laboratories (Dr. John Melnyk, Department of Biology, City of Hope Medical Center, Duarte, California and Mrs. Mary Jane Webb, State of Idaho Department of Health, Laboratories Division, Statehouse, Boise, Idaho).

Culture of Cells and Preparation of Slides

a. Peripheral blood lymphocytes

Lymphocytes from heparinized peripheral blood were cultured for varying lengths of time (6-72 hours) by the method of Moorhead et al. (1960). A mitotic inhibitor was not added to the majority of the cultures. In instances where a mitotic inhibitor was used, two to three drops of colchicine (Lilly) concentration = 10 mg/ml, were added per culture.

Modifications in the treatment of peripheral blood cells were as follows:

1) Cells were cultured in standard minimum essential medium, Eagle (Gibco) to which fetal calf serum (12 percent), l-glutamine
(0.3 percent), penicillin and streptomycin (1000 units/ml) and phytohemagglutinin (PHA) (0.1 μg/ml) (Burroughs Welcome MR-10) were added.

2) Cells from freshly drawn blood were not subjected to culture but otherwise were treated in the same fashion as were the cultured cells (see fixation).

3) Lymphocytes were separated from other types of peripheral blood cells on a Ficol-Isopaque density gradient according to the method of Thorsby and Bratlie (1970), and subsequently cultured in standard culture medium as described in Step 1.

4) Lymphocytes were separated from other types of peripheral blood cells as described in Step 3 and were not subjected to culture but were handled according to Step 2.

Both cultured and non-cultured cells were handled in the same way for slide preparation. They were washed in balanced salt solution (Hanks), centrifuged at 800 RPM, resuspended in hypotonic 0.075 M KCl solution for five minutes, recentrifuged and fixed in several changes of methanol-acetic acid (3:1). The cells were then resuspended in fresh fixative, dropped onto cold wet slides and allowed to air dry.

The conditions of culture were kept as uniform as possible when the results of cultures were to be compared as in the cell growth and labeling experiments.

For lymphocyte cultures, the plasma and buffy coat (containing lymphocytes and other white blood cells) and approximately ¼ cc of
erythrocytes were drawn off from approximately 30 cc of settled blood and mixed thoroughly. PHA, fetal calf serum and penicillin and streptomycin were added to 100 ml of culture medium which was mixed thoroughly and distributed in equal amounts to culture vials (5 ml/vial). One ml of the cell suspension was then added to each culture vial. Culture vials were shaken at periodic intervals during the incubation period to prevent localized clumping of cells.

b. Cultured fibroblasts

Skin biopsies were clinically obtained from five subjects: Individual I (XY); Individual IV (XYY); Individual V (XXYY); Individual VI (XYq+); Individual VII (XXY). These were placed in Gibco F-10 culture medium plus 16 percent fetal calf serum and penicillin and streptomycin, and transported to the laboratory. The biopsies were then rinsed in several changes of balanced salt solution under sterile conditions and cut into explants approximately 1 mm square. The explants were placed into disposable culture flasks with enough culture medium to barely cover the bottom of the flask. The flasks were then purged with a 95 percent air and five percent CO\textsubscript{2} mixture and incubated at 37° C. In three to four weeks after the explants had formed a confluent layer of cells over the bottom of the flasks, the cells were trypsinized from the bottom of the flask (0.5 percent trypsin and 0.2 percent EDTA) and transferred to new flasks. In most cases the cells were allowed to go through two or three trypsin transfers before they were harvested.

Cells which were to be harvested were allowed to grow six to 48
hours after the last transfer. In some cases the cells were grown directly on coverslips or slides, then treated with hypotonic solution (one part BSS:15 parts distilled water) and fixed. In other cases the cells were trypsinized from the flask, washed with balanced salt solution, spun down and resuspended in hypotonic solution and fixed in methanol-acetic acid (3:1). The cells were then resuspended in fixative and dropped onto cold, wet slides and allowed to air dry.

For serial cultures of fibroblasts, the cells of a confluent culture were trypsinized and the number of cells in the suspension determined using a hemocytometer. Equal amounts of the suspension were then transferred to new flasks or plated out on slides in covered sterile Petri dishes. In the latter case the cells were allowed to settle out and attach to the slides before additional media was added.

c. Hair root sheath cells

Hairs were plucked from the top of the head of subjects and placed in covered Petri dishes containing filter paper dampened with balanced salt solution. Sheaths were then dissected from the hair shaft in a drop of saline on a slide, or in a drop of glacial acetic acid. Sheaths fixed in acetic acid were squashed with a coverslip which was then removed, and the preparation allowed to air dry. Sheaths dissected in saline were transferred to several drops of trypsin-EDTA solution for one-half hour, fixed with methanol-acetic acid (3:1), dropped onto cold wet slides and allowed to air dry. Slides were stored at room temperature until studied.
d. Buccal mucosal cells

Buccal smears were taken from several of the subjects and fixed either in one part absolute ethanol:one part ethyl-ether (Papanicoli's fixative) or sprayed with Spray-Cite (Adams) and allowed to air dry. Smears fixed overnight in Papanicoli's fixative were then transferred to 95 percent ethanol until ready for use.

Variations in Hypotonic Treatment and Fixation of Cells

Several methods of fixation were tried on both fibroblasts and lymphocytes. The majority of lymphocytes were treated first with 0.075 M KCl for five minutes as described previously. Lymphocyte cultures from Individual I, however, were divided in half. One half of each culture was treated with the preceding hypotonic treatment and fixed in methanol-acetic acid (3:1). The other half was not treated with hypotonic solution but fixed in one of several ways: 1) cells were suspended in balanced salt solution (Hanks) and a drop or two of the suspension was drawn out on a clean dry slide as in making blood smears. Slides prepared in this way were allowed either to air dry or to dry partially and then were fixed for ten minutes in a) methanol-acetic acid (3:1), b) ethanol-acetic acid (3:1), c) 100 percent ethanol, or d) 100 percent methanol; 2) the remainder of the suspension was fixed in several changes of methanol-acetic acid, dropped onto cold wet slides, and allowed to air dry as for slides which had been treated with hypotonic solution. Slides were stored at room temperature until studied. Fixatives containing
formalin were avoided because of the apparent blocking action to QM staining (Linder, personal communication).

Several methods of fixation with and without hypotonic treatment were also applied to fibroblast cells. Cells from four individuals were grown directly on slides. Several slides from the same culture were then treated as follows: 1) cells were treated with hypotonic solution (15 parts distilled water: one part Puck's saline) for one-half hour, then fixed in methanol-acetic acid (3:1), 2) cells were fixed as in Step 1 but without hypotonic treatment, 3) cells were fixed for ten minutes in a saturated solution of HgC1₂ + five percent acetic acid (sublimate-acetic), then rinsed in several changes of 70 percent ethanol followed by a one hour treatment in 70 percent ethanol-I₂ solution. The slides were then passed through the alcohol series to the staining solution.

Quinacrine Staining and Fluoromicroscopy

Quinacrine staining of cells was done according to the method of Caspersson et al. (1970a). The cells were stained in a 0.5 percent solution of QM made up in McIlvane's (phosphate-citric acid) buffer at pH 7.0 for 20-30 minutes. The preparations were rinsed in several changes of the same buffer and sealed in buffer with a coverslip and nail polish, and then photographed within 24 hours after staining.

Photography

A Zeiss photomicroscope II equipped with a 35 mm camera base,
with conventional and phase optics, was used in photography. For QM stained material, a HB200 mercury light source with BG-12 excitation filter and a 530 nm barrier filter was used. Cells were photographed under reflected light on Kodak Tri-X panchromatic film and developed in Kodak D-76 developer.

Aceto-orcein stained material was photographed using phase optics.

DNA-synthesis and Autoradiography

Lymphocyte cultures were labeled with tritiated thymidine \((^{3}H\text{-TdR}, \text{New England Nuclear, specific activity 2.0 c/mM})\) in order to study the DNA synthesis of the Y-body in its various morphological configurations. Peripheral blood cell cultures were grown from 60-72 hours and \(^{3}H\text{-TdR}\) was added for varying lengths of time at a concentration of one \(\mu c/ml\) of culture medium.

Length of the labeling times selected were partly based on previous knowledge (Miller, 1970; Priest, 1969) of the duration of the various stages of the cell cycle. The length of the S period in lymphocytes has been generally determined to range from 6-12 hours; the length of G-2, from 3-5 hours; the length of G-1, from 6-24 hours.

Labeling studies fell into the following categories:

a. Continuous terminal labeling without colchicine

Cells were labeled during the culture period between 60 and 73 hours when the greatest number of cells were presumed to be in the S-period of the cell cycle. \(^{3}H\text{-TdR}\) was added at 60 or 66 hours for
3, 10, 15, 20, 30 or 80 minutes, and for 3, 5, or 13 hours, after which the cells were washed with balanced salt solution, treated with 0.075 M KCl for five minutes and fixed in several changes of fixative.Slides were prepared in the usual way. The slides were stained with QM and 150-200 nuclei showing the Y-body in its various morphological configurations were photographed from each culture. The morphology of the Y-body was scored from the photographs as to whether it was condensed (Stage II), dispersed (Stage III), highly dispersed (Stage IV) or in G-2 (prophase) (Stage V). A fifth category included nuclei which were ≤ 20 μ (Stage I), and non-transformed in appearance; nearly all of these nuclei had a condensed Y-body, and were subsequently classified as being in G-1. Cells which were in prophase (Stage VI) and metaphase (Stage VII) were also photographed from each culture.

After the nuclei were photographed by fluorescent microscopy, the slides were restained with aceto-orcein, stripped with Kodak AR-10 stripping film and stored in the dark. Depending on the length of time that the cultures were labeled, the slides were exposed for two and one-half to 12 days. At the end of that period the slides were developed and the nuclei relocated and photographed by phase contrast microscopy. The diameter of each nucleus was also measured.

Grain counts on the Y-body and over equivalent areas of the nucleus were done from separate photographs of the same nuclei. To obtain grain counts, the outlines of the QM stained nucleus and the Y-body were traced on a sheet of celluloid. The outline tracing was
then realigned over the photograph of the labeled nucleus and the silver grains within the outline of the Y-body counted. To obtain grain counts over an equivalent area of the nucleus, the approximate center of the nucleus was found with a thumbtack. The traced outline was then rotated 90°, 180° and 270° over the photograph of the labeled cell, using an underlying sheet of graph paper as a reference. Grains within the outline of the Y-body were counted at each of these positions and the mean of the three counts taken to represent the labeling of an "equivalent part of the nucleus".

b. Pulse-chase studies

Cultures, set up at the same time under identical conditions, were incubated for 61 hours at which time $^3$H-TdR was added to the cultures for a three hour period. At the end of the labeling time the pulse labeled cultures were washed with cold thymidine at a concentration 10X that of the tritiated thymidine to remove residual label. They were reincubated with fresh medium for additional lengths of time; 3, 6 and 9 hours. The inhibitory effect on cells during their growth period produced by a complete change of medium was avoided by removing half of the medium (preconditioned) from the cultures and replacing it with fresh medium containing $^3$H-TdR. After washing the label out of the cultures with cold thymidine, this preconditioned medium was mixed with an equal amount of fresh medium (both prewarmed to 37° C.) and added to the cultures for the final incubation period. One culture was fixed immediately. The remaining cultures were fixed at 67, 70 and 73 hours respectively. Cells with
different Y morphology from each of the cultures were photographed and the slides stripped at the same time with autoradiographic emulsion, exposed for six days, and developed simultaneously. The labeled cells were then rephotographed and the grains counted over a portion of each nucleus by marking a $20^\circ$ sector on a piece of celluloid. The radius of each nucleus was then determined using a millimeter scale scored along one leg of the sector. The grains over the nucleus were counted within the $20^\circ$ sector which formed the two radii. The mean number of grains over nuclei representing the various Y morphologies from each culture were thus determined.

c. Continuous terminal labeling with colchicine arrest

Cultures were labeled for the last six hours of the culture period. Colchicine was added two hours before harvesting the cells. Metaphases of such cultures were analyzed by autoradiography to check the labeling pattern of Y chromosomes (Schmidt, 1963).
III. RESULTS

Morphology of the Y-body at Interphase

The Y-body was detectable in interphase nuclei as a brightly fluorescent body in all 15 of the individuals studied. In the majority of cells this bright body was a discrete spot often located at the periphery of the nucleus in contact with the nuclear membrane. In a high percentage of cells of individuals with more than one Y chromosome (i.e. XYY and XYYY) the number of such spots was equivalent to the number of Y chromosomes. The size of the Y-body or bodies and the ease of detection varied considerably from individual to individual. This variation in size corresponded directly with the length of the fluorescent segment of the Y chromosome in metaphases examined from the same individuals. This correlation was particularly evident in Individuals I, V and VI who had striking differences in the size of their Y body (Plate I).

In addition to variations in size, the Y-body or bodies were observed to be in various morphological configurations within nuclei of the same individual. These configurations were of particular interest and were studied in considerable detail.

a. In peripheral blood lymphocytes

Several hundred to several thousand lymphocyte nuclei were examined microscopically or were photographed from each subject. The Y-bodies in cultured lymphocytes displayed various morphological configurations (Plate II). These ranged from a highly condensed
bright spot in small, uniformly fluorescent nuclei to what appeared as varying degrees of dispersion of fluorescence of the Y-body in larger nuclei stimulated by PHA, to a partially condensed configuration in those nuclei which appeared about to enter into prophase as judged by chromatin condensation. In the latter nuclei the Y-bodies often resembled the Y chromosome in metaphase. The configurations appeared to be correlated with the size and stage of the cell cycle of the interphase nucleus.

Non-cultured lymphocytes, not stimulated with PHA, were also examined from several of the subjects for the presence of the dispersed fluorescence of the Y-body. The cells were generally small and non-transformed (non-blastic) in appearance and the frequency of the dispersed fluorescence was much lower.

The dispersed fluorescence of the Y-body seen in cultured lymphocytes was more easily detectable in some individuals, particularly in Individual I having Yq+ (Plates I and II).

b. In fibroblasts

Similar configurations of the Y-body or bodies were seen (Plate III) in cultured fibroblasts from all individuals studied. As in the lymphocytes, the Y-body was visible in all stages of the cell cycle. Since the fibroblast nuclei were more irregular in size and shape, no readily apparent correlation of Y morphology with nuclear size was evident.

c. In buccal mucosa and hair root sheath cells

The Y-body was studied in buccal mucosa cells and hair root
sheath cells from several individuals including the individual with Yq+. The Y-body appeared only in the condensed configuration in all of the cells which were examined. No cells were observed to be undergoing mitosis.

**Occurrence of More Than One Y-body**

The morphology of the Y-bodies in lymphocyte and fibroblast nuclei from the six subjects having more than one Y chromosome was examined. Individuals VIII (with XYY) and IX (with XXYY) were particularly favorable for study. In 91.5 percent of their lymphocyte nuclei both of the Y-bodies present in a single nucleus showed identical configurations; either condensed or dispersed to the same degree. Eight-and-one-half percent of the nuclei had Y-bodies in different morphological configurations.

The separation of the Y-bodies varied from the maximum, i.e., at opposite sides of the nucleus, to being very close together (Plate V). In the latter extreme, these were often touching one another or only slightly separated. The Y-bodies, however, were the same size whether close together or far apart. Occasionally nuclei were seen in which one of the Y-bodies appeared cleaved producing two smaller bodies, the other Y-body being of normal size. In some cases a single Y-body present in the nucleus appeared larger than usual suggesting that two Y-bodies in close proximity appeared as one mass (Plate V).
Effect of Varying Hypotonic and Fixation Procedures

Fixation appeared to have little effect on the morphology of the Y-body in interphasic lymphocytes. The various fluorescent configurations of the Y-body were present regardless of the treatment and type of fixation used. In general, the addition of acetic acid to the fixative appeared to effect a more intense fluorescence than fixation with alcohol alone.

The treatment of lymphocytes with hypotonic 0.075 M KCl prior to fixation gave superior nuclear morphology. Cells fixed without hypotonic treatment often appeared crenulated indicating considerable shrinkage and clumps of fluorescent nucleoplasm often obscured the Y-body entirely. Despite the poorer morphology of non-hypotonic treated nuclei, the configurations of the Y-body were still evident in many of the cells. KCl treatment appeared to compensate for the shrinkage observed in non-hypotonic treated cells.

Fixation appeared to have even less effect on the morphology of the Y-body in fibroblast nuclei. Cells grown directly on slides and fixed in various ways gave identical results with respect to the morphology of the Y-body. Although cells treated with hypotonic solution before fixation appeared slightly more swollen (Plate IV) than non-hypotonic fixed cells (Plate III), there was no apparent disruption of the structure of the Y-body. Instead the treatment with hypotonic solution appeared to make the Y-body more distinct from the surrounding nucleoplasm. HgCl$_2$-acetic acid fixation (Plate IV) produced better preservation of the cytoplasm than
methanol-acetic acid fixation and thus is responsible for a slightly higher background fluorescence. This higher background did not interfere with detection of the Y-body since there was less clumping of the nucleoplasm.

Fixation did appear to have an effect on the double structure described by other investigators. The dual structure was more frequent in non-hypotonic treated cells than in cells fixed after hypotonic treatment.

**Relationship of Y-body Morphology to Nuclear Size and Cell Transformation**

Slides from the various cell preparations were scanned at predetermined intervals under the microscope. Nuclei were measured as they appeared in the field. The criteria for selection were 1) the nuclei were reasonably spherical and isolated from other nuclei in the field, 2) the identification of the Y-body and its morphology was unambiguous, and 3) the Y-body could not be confused with other fluorescent debris or brightly fluorescent areas in the nucleus. The morphology or absence of the Y-body was noted for each nucleus measured.

The dispersion of the Y-body was examined in 1) non-stimulated, non-cultured mixed lymphocytes; 2) cultured mixed lymphocytes stimulated with PHA; 3) cultured pure lymphocytes stimulated with PHA; 4) non-cultured pure lymphocytes. The variability in Y-body morphology was most evident in PHA stimulated lymphocytes in culture.
The amount of dispersion appeared to be correlated with nuclear size and blastic transformation of cells induced by PHA.

Nuclei of several types were seen in the preparations. These types included 1) small, brightly fluorescent, uniform sized nuclei which did not show any sign of nuclear differentiation with the exception of the small highly condensed intensely fluorescent Y-body; 2) larger nuclei of more variable size which had a more diffuse, less brightly fluorescent nucleoplasm with the exception of the brightly fluorescent Y-body and other bright spots and clumps of nuclear material; 3) polymorphonuclear leucocytes. The proportions of these nuclear classes varied according to the preparation.

In non-stimulated, non-cultured preparations all three classes of nuclei were present. The polymorphonuclear leucocytes did not show evidence of dispersion of the Y-body, nor did the smaller uniform-sized lymphocytes. Evidence of dispersion was occasionally observed in the larger nuclei, some of which undoubtedly represented granulocytes. A high degree of dispersion was not seen in any of the non-cultured preparations.

Lymphocytes cultured and stimulated with PHA had a much higher proportion of larger nuclei of varying diameter. Dispersed Y-body morphology was seen in a high proportion of these nuclei. Polymorphonuclear leucocytes were not observed.

Non-cultured separated lymphocytes showed a uniform population of small condensed nuclei of highly uniform diameter. The Y-body in these nuclei was almost always in the condensed configuration (Plate VIa).
TABLE I. COMPARISON OF NUCLEAR SIZE, AND MORPHOLOGY AND FREQUENCY OF THE Y-BODY IN DIFFERENT PREPARATIONS OF CELLS FROM PERIPHERAL BLOOD FROM FOUR NORMAL MALES WITH 46,XY

<table>
<thead>
<tr>
<th>Individual</th>
<th>Morphology</th>
<th>Number of cells</th>
<th>Mean dia.</th>
<th>s.d.</th>
<th>Percent</th>
<th>Number of cells</th>
<th>Mean dia.</th>
<th>s.d.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>condensed</td>
<td>141</td>
<td>10.4 ± 2.8</td>
<td>87.0</td>
<td></td>
<td>147</td>
<td>13.8 ± 4.8</td>
<td>57.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dispersed</td>
<td>8</td>
<td>11.4 ± 1.5</td>
<td>5.0</td>
<td></td>
<td>86</td>
<td>22.2 ± 1.5</td>
<td>33.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>no Y-body</td>
<td>13</td>
<td>13.0 ± 2.3</td>
<td>8.0</td>
<td></td>
<td>22</td>
<td>15.2 ± 4.7</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>condensed</td>
<td>36</td>
<td>10.9 ± 6.0</td>
<td>81.8</td>
<td></td>
<td>21</td>
<td>17.1 ± 5.5</td>
<td>48.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dispersed</td>
<td>0</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>21</td>
<td>30.8 ± 6.1</td>
<td>48.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>no Y-body</td>
<td>8</td>
<td>12.5 ± 2.8</td>
<td>18.2</td>
<td></td>
<td>1</td>
<td>23.0 ± 5.9</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>III</td>
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<td>24</td>
<td>8.8 ± 1.5</td>
<td>80.0</td>
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<td>13</td>
<td>14.5 ± 3.5</td>
<td>59.1</td>
<td></td>
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<tr>
<td></td>
<td>dispersed</td>
<td>0</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>8</td>
<td>25.8 ± 2.3</td>
<td>36.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>no Y-body</td>
<td>6</td>
<td>10.0 ± 2.9</td>
<td>20.0</td>
<td></td>
<td>1</td>
<td>24.0 ± 3.8</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

|            |            |                 |           |      |         |                 |           |      |         |
| Separated Lymphocytes | | | | | | | | |
| IV          | condensed  | 37              | 11.3 ± 0.7 | 100  |         | 24             | 13.1 ± 3.4 | 46.2 |
|            | dispersed  | 0               | *           | *    | *       | 26             | 24.4 ± 3.8 | 50.0 |
|            | no Y-body  | *               | *           | *    | *       | 2              | 9.2 ± 3.8  | 3.8  |

* = not scored
Preparations of pure lymphocytes cultured in the presence of PHA showed a range of nuclear diameters and varied Y-morphology as was seen in the lymphocytes cultured in the normal way (Plate VIb).

The results of nuclear measurements and the comparisons of the percent of condensed versus dispersed Y-body morphology in the different cell preparations from four individuals are presented in Table 1.

**Nuclear Size Distribution**

The distribution of nuclear diameter with Y-body morphology was also studied in cultured lymphocytes from three individuals (I, V and VI), who had marked differences in the size of their Y-body (Plate I).

Nuclei from these three subjects were chosen as they appeared in the microscope field by the criteria previously described. The nuclear diameters were measured and the morphology of the Y-body noted. The nuclei were scored as to whether the Y-body was **condensed**, **dispersed**, **highly dispersed** or **not present**.

The measured nuclei were arbitrarily separated into classes by size. The number of nuclei with a particular Y-body morphology was expressed as a percent of the total number of nuclei within each size classification. The percentages presented in the bar graphs (Figure 1) show a similar distribution of nuclear size with Y-body morphology for each of the three individuals. In all three individuals there appeared to be an inverse relationship between percent of nuclei
Figure 1. Nuclear size distribution for the different configurations of the Y-body in three individuals with different sized Y-bodies (Plate I): a) Individual I with normal sized Y-body; b) Individual VI with Yq+; c) Individual V with small Y-body.

- □ condensed Y-body,
- □ dispersed Y-body,
- □ highly dispersed Y-body,
- □ no Y-body
having a condensed Y-body and increased nuclear diameter. In the 
Individuals I (Figure 1a) and VI (Figure 1b) there appeared to be a 
direct relationship between percent of nuclei having a dispersed 
Y-body and increased nuclear diameter. In the first two individuals 
the percent of nuclei with no Y-body appeared to be independent of 
nuclear size. In Individual V (Figure 1c) the frequency of nuclei 
with no Y-body increased somewhat in nuclei ≥ 29 microns in diameter, 
and there was a corresponding decrease in percent of nuclei with 
dispersed Y-body morphology in the same nuclear size classifications.

Frequency of the Y-body

In all of the cultures examined no Y-body was observed in a 
certain percentage of nuclei. The number of such nuclei varied from 
individual to individual and ranged from 2.4 percent to 45 percent 
(Table 1 and Figure 1). Some of the technical factors which 
contributed to difficulty in detecting the Y-body were 1) high back-
ground fluorescence which tended to obscure nuclear detail; 2) the 
presence of fluorescent debris and other fluorescent spots in the 
nucleus; 3) the small size of the Y-body in some of the subjects. 
A fourth possible factor was disappearance of the Y-body due to 
dispersion.

The data (Figure 1 and Table 1) indicate that dispersion of the 
Y-body was not a contributing factor to decreased frequency except 
when the Y-body was very small. In Individuals II and III (Table 1) 
the frequency was lower in nuclei in which the Y-body was seen only
in the condensed configuration. The Y-body of these two individuals was small and was more readily identifiable when located on the periphery of the nucleus. The high background fluorescence of the small brightly fluorescent nuclei may account for the apparent decrease in Y-body frequency.

The frequency of the Y-body in Individuals I, V and VI, who had notable differences in the size of their Y-bodies (Plate I and Figure 1), shows a direct relationship to the size of the Y-body. Individual V (Figure 1c), who had a very small Y-body, had from 25-45 percent of nuclei without a Y-body. Individual I (Figure 1a, Table 1) with an intermediate-sized Y-body had from 10 to 20 percent of nuclei without a Y-body. Individual III (Figure 1b), who had a large-sized Y-body, had the Y-body in approximately 98 percent of nuclei, suggesting that the Y-body was more consistently recognizable and rarely lost its identity due to dispersion.

The Y-body in the Cell Cycle

a. Lymphocytes

Eight lymphocyte cultures from Individual I were set up under similar conditions with the same number of cells inoculated into each culture and were harvested at six hour intervals from 0-48 hours. The harvested cells were treated with 0.075 M KCl and fixed as previously described. A control sample was treated without culturing.

Slides from each culture were stained with QM and scanned under
the fluorescent microscope. The diameters were measured and the morphology of the Y-body scored in 100-150 nuclei. The mitotic index (ratio of dividing to total cells) was also determined for each sample.

The frequency of the dispersed Y-body showed a direct relationship to the number of hours in culture. The proportion of cells with a condensed Y-body showed an inverse relationship to time in culture. The graph (Figure 2) shows the percentage of nuclei which had a dispersed, condensed, or no detectable Y-body plotted against hours in culture. The frequency of nuclei without a Y-body appeared to decrease somewhat with the decrease in frequency of nuclei with a condensed morphology, as previously described. Mitotic activity (Figure 2) was not evident until approximately 42 hours.

The results of the measurements of nuclear diameters for each of the cultures are shown in Figure 3. No distinction was made between dispersed and highly dispersed morphology in the scoring. As seen for the frequency of dispersed morphology, the diameter of nuclei with a dispersed Y-body showed a direct relationship to the number of hours in culture. A marked decrease in the diameter of nuclei with a dispersed Y-body at 48 hours suggests that the larger nuclei have gone into mitosis leaving a population of smaller nuclei behind. The small size of nuclei with a dispersed configuration at the beginning of the culture period suggests that dispersion in some nuclei begins almost immediately with cell transformation. In cultures harvested after 12 hours none of the small nuclei showed a highly dispersed Y-body.
Figure 2. Percent of lymphocyte nuclei having a condensed, dispersed, or no Y-body and mitotic indices of cultures harvested at six hour intervals from 0-48 hours.

Figure 3. Mean diameters and standard deviations of nuclei having condensed, dispersed or no Y-body from lymphocyte cultures harvested at six hour intervals from 0-48 hours.
Nuclei with a condensed Y-body showed almost no increase in nuclear diameter with time in culture, indicating that nuclei reaching a certain size begin to undergo dispersion of the Y-body. Nuclei with apparent absence of the Y-body were more variable in diameter, indicating that its absence was not restricted to a single size classification.

b. Fibroblasts

Serial cultures of fibroblasts were harvested from Individual VI, who had a large Y-body, in a manner similar to the lymphocyte cultures above. Fibroblasts from skin were grown in culture for 30 days and taken through two trypsin transfers. After the second transfer fourteen cultures were set up under identical conditions with an equal number of cells in each flask. The flasks were then harvested at six hour intervals from 0-48 hours and each day thereafter for eight consecutive days. Cultures were also harvested on the 10th, 12th, 15th, and 16th day. Cell number was again determined for each culture at the time of harvesting. Aliquots of the 30 and 48 hour cultures were plated out on slides and incubated until 36 and 54 hours, respectively.

Slides from each sample were scanned and 100 nuclei were scored as to the morphology of the Y-body. The mitotic index was also determined for each time.

The resulting growth curve obtained for the fibroblast cultures over the sixteen day period, the corresponding mitotic index, and the frequency of cells having a dispersed Y-body are presented in
Although cell numbers deviated somewhat from the expected theoretical curve for cell growth, the conventional pre-logarithmic, logarithmic and post-logarithmic phases are evident. In the overall trend, the highest frequency of Y-body dispersion was seen during the pre-log and log phases of growth with a gradual falling off of dispersion in the late log and post-log phases. A striking correlation between dispersion and cell number was seen in the early part of the growth curve (at 30 hrs) when a sudden unexplained drop in cell number occurred together with a corresponding drop in number of cells showing Y-body dispersion. At 54 hours a second drop was seen in the proportion of cells with a dispersed Y-body. However, at this point the number of cells was not determined so that there is no way of knowing if a corresponding change in the growth rate occurred at this point.

Dispersion of the Y-body showed a striking correlation with mitotic index at all points (Figure 4). This correlation was particularly evident at the beginning of the growth curve when the cells were presumably most highly synchronized and the time interval between harvests was shortest. The cultures were fed at six days. Twenty-four hours after feeding, this correlation was again evident, as reflected by increases both in mitotic activity and in dispersed morphology.

Based on evidence from both lymphocyte and fibroblast cultures, the data suggest that Y-body dispersion is closely related to the
Figure 4. Relationship of fibroblast cell growth, Y-body dispersion and mitotic index.
cell cycle and cell division and not to intrinsic differences in cell morphology.

**DNA-synthesis of the Y-body and the Y Chromosome**

a. Continuous terminal labeling without colchicine

Cultures of peripheral blood lymphocytes from Individual VI (Plate I-II) were set up under identical conditions and $^3$H-TdR added to the different cultures for various lengths of time.

Grain counts were done on approximately 1900 nuclei from twelve cultures. Cultures which were labeled for three minutes at 61 and 66 hours were combined as were cultures which were labeled for 10 minutes at the two times, since there appeared to be almost no difference in the distribution or amount of label for the different culture times in either instance. Likewise, grain counts from cultures labeled for 15 and 20 minutes at 61 hours of culture were combined for the same reason. The remaining cultures, which were analyzed, were labeled at 61 hours for 30 min., 80 min., 3 hrs., 5 hrs., and 13 hrs., respectively. The grain counts for Y-bodies with different morphology at different labeling times are given in Table 2. All grain counts were standardized on the basis of a six day exposure time. Therefore, corrections in grain counts were made for differences in time of exposure to the autoradiographic stripping film; grain counts from cultures exposed for 12 days were divided by two and grain counts from cultures exposed for 2.5 days were multiplied by a factor of 2.4. The mean diameters of the
nuclei and the percent of nuclei that labeled are also given.

The mean diameters of nuclei having a particular Y-body morphology varied somewhat from culture to culture. In all cultures there was increased dispersion of the Y-body with increasing mean nuclear diameter. Nuclei classified as being in G-2 (Stage V) tended to show the greatest variation in nuclear size.

The percent of cells which labeled per culture ranged from 27.5-86.4. The percent labeling tended to increase with longer labeling time for every nuclear class, the most marked changes occurring for those nuclei which were classified as G-1, G-2, or were clearly undergoing cell division. Nuclei classed as being in G-2 (Stage V) ranged from 24 percent to 100 percent labeled. The mean was 54.9 percent. The nuclei which showed the lowest percent labeling were those classified as being in G-1 (Stage I). The mean for these nuclei was 9.6 percent (range 0-36.8 percent). As might be expected, the highest percent of labeled cells in this class was from the culture exposed to $^3$H-TdR for the longest time (13 hours). The overall percent of labeled nuclei was consistently higher for the three remaining classes (Stages II-IV) of Y-body morphology. The mean percent of labeled nuclei > 20 microns in diameter with a condensed Y-body (Stage II) was 64.9 (range 49.0-80.6 percent); for nuclei with a dispersed Y-body (Stage III) the mean was 69.3 percent (range 50.8-90.9 percent); for nuclei with a highly dispersed Y-body (Stage IV) the mean was 68.6 percent (range 47.2-95.8 percent).

These data tend to confirm the classifications that were made with regard to Y-body morphology and suggest that nuclei > 20 microns
TABLE 2. RESULTS OF GRAIN COUNTS OVER THE Y-BODY IN DIFFERENT MORPHOLOGICAL CONFIGURATIONS AND IN DIFFERENT STAGES IN THE CELL CYCLE, AND OVER AN EQUIVALENT AREA OF THE NUCLEUS. THE TABLE ALSO SHOWS MEAN DIAMETERS AND PERCENT OF CELLS WHICH WERE LABELED.

<table>
<thead>
<tr>
<th>Morphology or Stage</th>
<th>Labeling Time</th>
<th>No. of cells</th>
<th>No. grains over Y-body</th>
<th>No. grains over equiv. nuclear area</th>
<th>Nuclear diameter</th>
<th>Percent nuclei labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3'</td>
<td>69</td>
<td>0.8 ±0.7</td>
<td>0.3 ±0.4</td>
<td>16.3 ±2.3</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>10'</td>
<td>56</td>
<td>0.7 ±0.6</td>
<td>1.1 ±0.8</td>
<td>17.0 ±2.4</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>15 &amp; 20'</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>16.8 ±2.4</td>
<td>0</td>
</tr>
<tr>
<td>Condensed</td>
<td>30'</td>
<td>30</td>
<td>1.4 ±1.3</td>
<td>2.3 ±1.6</td>
<td>17.5 ±2.3</td>
<td>16.7</td>
</tr>
<tr>
<td>≤20µ</td>
<td>80'</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>17.4 ±2.9</td>
<td>0</td>
</tr>
<tr>
<td>(G-I)</td>
<td>5 hr</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>18.4 ±2.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5 hr</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13 hr</td>
<td>19</td>
<td>19.7 ±18.9</td>
<td>15.6 ±13.2</td>
<td>17.8 ±1.9</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.6</td>
</tr>
<tr>
<td>II</td>
<td>3'</td>
<td>97</td>
<td>1.4 ±1.4</td>
<td>1.2 ±1.0</td>
<td>27.3 ±2.3</td>
<td>62.9</td>
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<tr>
<td></td>
<td>10'</td>
<td>121</td>
<td>2.1 ±2.4</td>
<td>2.3 ±1.6</td>
<td>27.3 ±2.3</td>
<td>62.0</td>
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<tr>
<td></td>
<td>15 &amp; 20'</td>
<td>104</td>
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<td>3.1 ±2.7</td>
<td>28.2 ±2.5</td>
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</tr>
<tr>
<td>Condensed</td>
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<td>103</td>
<td>4.5 ±4.5</td>
<td>5.0 ±3.1</td>
<td>29.7 ±2.5</td>
<td>64.1</td>
</tr>
<tr>
<td>≥20µ</td>
<td>80'</td>
<td>66</td>
<td>6.8 ±5.4</td>
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<td>46</td>
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<tr>
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<td>5 hr</td>
<td>58</td>
<td>11.0 ±11.8</td>
<td>10.9 ±11.7</td>
<td>30.0 ±5.1</td>
<td>67.2</td>
</tr>
<tr>
<td></td>
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<td>62</td>
<td>17.5 ±11.5</td>
<td>17.0 ±9.2</td>
<td>28.9 ±4.8</td>
<td>80.6</td>
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<tr>
<td>Mean Percent</td>
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<td></td>
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<td></td>
<td>64.9</td>
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<tr>
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<td>3'</td>
<td>49</td>
<td>1.6 ±2.4</td>
<td>1.2 ±1.2</td>
<td>29.9 ±2.5</td>
<td>59.2</td>
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<tr>
<td></td>
<td>10'</td>
<td>65</td>
<td>3.5 ±4.5</td>
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<td>30.6 ±2.5</td>
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<td>15 &amp; 20'</td>
<td>51</td>
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<td>34.4 ±2.6</td>
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<tr>
<td>Dispersed</td>
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<td>68</td>
<td>5.6 ±4.8</td>
<td>5.1 ±3.6</td>
<td>34.1 ±2.5</td>
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<td>80'</td>
<td>26</td>
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<td>5 hr</td>
<td>28</td>
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<td>15.8 ±16.2</td>
<td>37.6 ±5.6</td>
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<td>13 hr</td>
<td>33</td>
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<td>90.9</td>
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<td></td>
<td>69.3</td>
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<tr>
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<td>48</td>
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<td>2.6 ±2.5</td>
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<td>10'</td>
<td>72</td>
<td>4.0 ±4.3</td>
<td>3.2 ±2.6</td>
<td>35.2 ±2.6</td>
<td>47.2</td>
</tr>
<tr>
<td></td>
<td>15 &amp; 20'</td>
<td>76</td>
<td>11.1 ±11.4</td>
<td>6.1 ±5.0</td>
<td>37.4 ±2.6</td>
<td>63.2</td>
</tr>
<tr>
<td>Highly</td>
<td>30'</td>
<td>59</td>
<td>10.9 ±10.0</td>
<td>6.9 ±5.0</td>
<td>36.6 ±2.2</td>
<td>66.1</td>
</tr>
<tr>
<td>Dispersed</td>
<td>80'</td>
<td>53</td>
<td>24.3 ±21.7</td>
<td>17.2 ±11.3</td>
<td>39.6 ±2.6</td>
<td>77.4</td>
</tr>
<tr>
<td>3 hr</td>
<td>29</td>
<td>36.3 ±16.5</td>
<td>20.9 ±9.9</td>
<td>40.3 ±5.0</td>
<td>75.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 hr</td>
<td>21</td>
<td>63.4 ±21.9</td>
<td>43.5 ±13.9</td>
<td>39.6 ±5.4</td>
<td>71.4</td>
</tr>
<tr>
<td></td>
<td>13 hr</td>
<td>24</td>
<td>59.2 ±20.2</td>
<td>42.5 ±17.2</td>
<td>42.4 ±5.1</td>
<td>95.8</td>
</tr>
<tr>
<td>Mean Percent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>68.6</td>
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* Not determined
<table>
<thead>
<tr>
<th>Morphology or Stage</th>
<th>Labeling Time</th>
<th>No. of Cells</th>
<th>No. grains over Y-body</th>
<th>No. grains over equiv. nuclear area</th>
<th>Nuclear Diameter</th>
<th>Percent Labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>3'</td>
<td>23</td>
<td>2.0 ± 2.2</td>
<td>1.2 ± 1.3</td>
<td>33.7 ± 13.1</td>
<td>56.5</td>
<td></td>
</tr>
<tr>
<td>10'</td>
<td>30</td>
<td>1.8 ± 2.1</td>
<td>0.7 ± 1.8</td>
<td>32.2 ± 7.2</td>
<td>36.7</td>
<td></td>
</tr>
<tr>
<td>15 &amp; 20'</td>
<td>25</td>
<td>5.8 ± 2.7</td>
<td>2.3 ± 2.5</td>
<td>37.6 ± 10.4</td>
<td>24.0</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>30'</td>
<td>3.5 ± 3.6</td>
<td>3.0 ± 2.4</td>
<td>38.7 ± 7.4</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>G-2</td>
<td>80'</td>
<td>4.1 ± 2.6</td>
<td>1.7 ± 1.7</td>
<td>44.6 ± 8.9</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>Pre-prophase</td>
<td>3 hr</td>
<td>18.6 ± 15.8</td>
<td>8.6 ± 4.9</td>
<td>46.1 ± 5.6</td>
<td>77.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 hr</td>
<td>24.0 ± 11.8</td>
<td>10.8 ± 5.2</td>
<td>*</td>
<td>45.0</td>
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</tr>
<tr>
<td></td>
<td>13 hr</td>
<td>49.9 ± 22.8</td>
<td>29.5 ± 16.2</td>
<td>43.2 ± 5.7</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>3'</td>
<td>*</td>
<td>0</td>
<td>*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10'</td>
<td>*</td>
<td>0</td>
<td>*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 &amp; 20'</td>
<td>*</td>
<td>0</td>
<td>*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Prophase</td>
<td>80'</td>
<td>1.8 ± 3.9</td>
<td>0.8 ± 0.7</td>
<td>*</td>
<td>40.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 hr</td>
<td>10.9 ± 9.1</td>
<td>5.0 ± 4.1</td>
<td>*</td>
<td>93.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 hr</td>
<td>10.1 ± 8.7</td>
<td>6.2 ± 3.5</td>
<td>*</td>
<td>58.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 hr</td>
<td>28.8 ± 10.5</td>
<td>19.8 ± 4.0</td>
<td>*</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>3'</td>
<td>*</td>
<td>0</td>
<td>*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10'</td>
<td>*</td>
<td>0</td>
<td>*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 &amp; 20'</td>
<td>*</td>
<td>0</td>
<td>*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Metaphase</td>
<td>80'</td>
<td>*</td>
<td>0</td>
<td>*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 hr</td>
<td>8.8 ± 7.0</td>
<td>5.0 ± 2.7</td>
<td>*</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 hr</td>
<td>14.0 ± 8.8</td>
<td>7.1 ± 3.3</td>
<td>*</td>
<td>42.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 hr</td>
<td>33.9 ± 11.0</td>
<td>22.3 ± 7.3</td>
<td>*</td>
<td>88.2</td>
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<td>Total Cells = 1933</td>
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<td></td>
<td></td>
<td>Mean Percent 46.6</td>
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</table>

**TABLE 2. (Continued)**

Mean Percent of Labeled Cells/Culture for All Classes of Y-Body Morphology

<table>
<thead>
<tr>
<th>Label Time</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'</td>
<td>34.0</td>
</tr>
<tr>
<td>10'</td>
<td>30.4</td>
</tr>
<tr>
<td>15 &amp; 20'</td>
<td>27.5</td>
</tr>
<tr>
<td>30'</td>
<td>44.8</td>
</tr>
<tr>
<td>3 hr</td>
<td>52.4</td>
</tr>
<tr>
<td>5 hr</td>
<td>62.9</td>
</tr>
<tr>
<td>13 hr</td>
<td>84.6</td>
</tr>
</tbody>
</table>

* Not determined
in diameter, excluding G-2, were actually in the S-period. The fact that 100 percent of these cells did not label in any of the cultures would indicate that some cells did not take up label either because they were non-viable or because their ability to incorporate thymidine was in some way impaired. The mean grain counts given in Table 2 are for labeled cells only.

The mean grain counts obtained (Table 2) for the Y-body in its different morphologies and for an equivalent area of the nucleus have been plotted against labeling time in Figures 5 and 6. The Y-body increased in area as it became more dispersed and had a smaller area again in nuclei which were classified as being in G-2. No attempt was made to correct for this difference in size in the calculation of mean grain counts for the different morphologies. Thus changes in slope of the graphs are more important than the absolute numbers of grains depicted.

The labeling times, as depicted in Figures 5 and 6, can be divided into approximately three informative categories: 1) short labeling time (3-30 min.) during which labeled cells would be expected not to have moved very far and so would be closest to the stage they were in when label was introduced to the culture; 2) intermediate labeling time (1½-5 hrs.) during which cells which were in the S-period at the time label was added would be expected to take up label at their maximum rates; 3) long labeling time (5-13 hrs.) during which cells would have taken up the maximum amounts of label possible for the conditions of a particular culture,
dependent upon the proportions of cells which were at different stages in the cell cycle.

The distribution of label over the various classes of Y-body morphology in Figures 5 and 6 will be discussed in terms of the above categories of labeling time.

**Short labeling time.** With very short labeling times minor differences in the labeling activities of the various classes of nuclei are evident. Stage II-IV took up greater amounts of label than the remaining classifications of nuclei (Stage I or V), indicating that these nuclei actually were in the S-period (Figure 5).

The slight fluctuations in labeling activity seen in Stage I is less significant when it is considered that only a small percentage of these nuclei actually labeled. After 30 minutes, no label was seen in these nuclei, indicating that some were either misclassified or moved rapidly into the next stage. Similarly nuclei classed as being in G-2, Stage VI (Figure 6a) showed only small amounts of label, both over the Y-body and over an equivalent area of the nucleus.

With the short time labeling, there was already increased rate of DNA synthesis by the Y-body as compared to the rest of the nucleus. This more rapid rate of synthesis is particularly evident for the highly dispersed Y-body, less evident for the intermediately dispersed Y-body, and not evident at all for the condensed Y-body. Nuclei classed as G-2 consistently showed heavier labeling over the Y-body than over the nucleus, but there was no apparent difference in rate of label uptake between the two.
Intermediate labeling time. With longer labeling time the relative amounts of label taken up by the various classes of nuclei became more evident. There was, in general, an increase in slope of the graphs proportional to the degree of dispersion of the Y-body (Figure 5). This increase in slope is most readily explained if the stages represented are sequential. Nuclei which have been in the S-period the longest time will have accumulated the most label, since they will have accumulated label not only at the stage they were in when the culture was terminated, but from any preceding stages that they have passed through during the labeling period.

The relative times at which the various stages have reached maximum labeling are further evidence that they are sequential. The maximum rate of labeling for Stage II (Figure 5) was between 3 and 30 minutes after which it declined. A comparable decline for Stage III was not seen until approximately 80 minutes. No decline was seen for Stage IV until after five hours.

The rate of labeling showed a direct relationship to degree of dispersion of the Y-body. Nuclei with a condensed Y-body showed little or no difference in label over the Y-body compared to label over the equivalent part of the nucleus. With increased dispersion the amount and rate of labeling over the Y-body was increasingly greater than over the rest of the nucleus. This differential rate of labeling was particularly evident for Stage IV between 80 minutes and 3 hours when the rate of labeling over the rest of the nucleus appeared to have declined. In general, the differences in amount
Figure 5. Nuclei in G-1 and S periods of the cell cycle, showing mean number of number of grains over the Y-body and over an equivalent area of the nucleus (ordinate) with labeling time (abscissa). Sequence of stages and morphology of the Y-body are indicated by roman numerals: I, nuclei with condensed Y-body ≤ 20μ diameter (classified as G-1); II, nuclei with condensed Y-body > 20μ diameter; III, nuclei with dispersed Y-body; IV, nuclei with highly dispersed Y-body. The abscissa scale has been reduced (irregular line) between 5 and 13 hours for convenience in presentation.
Figure 6.  a) Similar representation to Figure 5 for nuclei classified as being in G-2 (Stage V).  Note approximate 80 minute lag in appreciable uptake of label compared to stages represented in Figure 5.

b) Similar representation for nuclei in prophase (Stage VI).  Note approximate 80 minute lag before any label appears in prophases.

c) Similar representation for cells in metaphase (Stage VII).  Note approximate lag of between 80 minutes and three hours before label is incorporated.
\[40-I = \text{mean no. of grains over Y-body}\]
\[\text{mean no. of grains over equivalent area of nucleus}\]

A

- \(\bullet\bullet\) = mean no. of grains over Y-body
- \(\triangle-\triangle\) = mean no. of grains over equivalent area of nucleus

B

C

V

VI

VII

Number of grains

30

20

10

80 Min

3 Hr

5

13 Hr
of label over the Y-body suggests a lag in labeling of the condensed Y-body compared to the rest of the nucleus and a subsequent faster rate of labeling as the Y-body becomes more dispersed.

The labeling behavior of Stage V depicted in Figure 6a confirms the classification of these nuclei selected as being in G-2. As might be expected if these cells were actually in G-2, there was an appreciable lag in significant labeling of these nuclei until approximately 80 minutes (approximately one-half the length of the G-2 period). Similarly there was an approximate 80 minute lag before any label appeared in prophase nuclei (Figure 6b), and a 3 hour lag before label appeared in metaphase cells (Figure 6c). The greater amount of label over the Y-body than over an equivalent area of the nucleus in all three stages is consistent with the knowledge the Y chromosome is late labeling.

The representative labeling behavior of the different stages of morphology for the different labeling times are demonstrated (Plates VII-XII).

Long labeling time. After 13 hours of labeling the mean number of grains over Stage IV showed a marked levelling off. In contrast, nuclei in Stage I showed a ten-fold increase in label. The levelling off in amount of label over the highly dispersed Y-body indicates that these nuclei were toward the end of the S-period and had already attained maximum labeling after five hours. The remaining classes of nuclei showed increases in amount of label comparable to those seen during the first five. This indicated that these nuclei represented
stages toward the middle or beginning of the S-period and that the majority of cells were either still in S at the time the culture was terminated at 13 hours or entered into S during the 13 hours.

b. Pulse-chase studies

Utilizing cultures from an Individual I with a normal sized Y-body, an additional 387 nuclei were analyzed from serial cultures which had been pulse-labeled. The results (Figure 7) show the distribution of label over the various classes of nuclei from the different cultures over the 12 hour period.

Since the labeling time, exposure to the autoradiographic emulsion, and development of the silver grains were the same for every culture, the distribution of label was dependent upon 1) the number of cells that were in a particular stage when the label was added, 2) whether the proportion of cells in different stages was the same for all the cultures at the time label was added, and 3) how far the cells in each of the cultures had moved through the cell cycle by the times that the cultures were terminated. The culture which was terminated the earliest would be expected to have cells closest to the stage that they were in when the label was added. With subsequently longer incubation times, assuming that the same relative numbers of cells were moving through the cell cycle at the same relative rates, progressively larger amounts of label would be expected to appear in sequentially later stages of the cell cycle.

The results (Figure 7) confirmed the above expectations. The culture which was terminated immediately following the three hour
b. Results of grain counts over pulse-labeled nuclei representing different morphological configurations of the Y-body. Roman numerals indicate different classes of nuclei in their proposed sequence: I = nuclei ≤ 20μ diameter; II = nuclei > 20μ diameter with condensed Y-body; III = dispersed Y-body; IV = highly dispersed Y-body; V = nuclei classed as G-2; V\textsuperscript{I} = prophase; VII = metaphase and later.

Figure 7a. Diagram of pulse labeling procedure.

b. Results of grain counts over pulse-labeled nuclei representing different morphological configurations of the Y-body. Roman numerals indicate different classes of nuclei in their proposed sequence: I = nuclei ≤ 20μ diameter; II = nuclei > 20μ diameter with condensed Y-body; III = dispersed Y-body; IV = highly dispersed Y-body; V = nuclei classed as G-2; V\textsuperscript{I} = prophase; VII = metaphase and later.
labeling period showed highest labeling over nuclei having been previously classified as being in the S period (Stages II-VI). With prolonged incubation, after pulse labeling, the amount of label over stages, classed as being in S decreases, and increases over successively later stages in the cell cycle.

The results from pulse labeling thus support those obtained with continuous labeling, namely that the different morphological configuration of the Y-body represented a progressively greater degree of dispersion during DNA synthesis and a subsequent recondensation of Y-body material as the nuclei enter into G-2.

c. Continuous terminal labeling with colchicine arrest

The more conventional technique of late labeling was done on 72 hour lymphocyte cultures from Individual VI to see if the labeling pattern of his long Y (Yq+) corresponded to the conventional labeling pattern of the Y chromosome from normal individuals (Schmid, 1963; Takagi and Sandbery, 1968). The Y chromosome heavily labeled in 12 of 19 metaphases which were examined and thus was consistent with the normal labeling pattern of the Y chromosome. This pattern was essentially the same as that obtained for metaphases from cultures which were continuously labeled for five hours without colchicine arrest (Plate XIII).
IV. DISCUSSION

Behavior of the Y-body in Interphase

At least part of the Y-body forms a discrete body of chromatin which fluoresces brightly in interphase when stained with quinacrine dyes. In the present study the fluorescent Y-body was observed to have variable morphology. The Y-body was seen to manifest this highly variable morphology in interphase nuclei both of fibroblasts and of peripheral blood lymphocytes. These configurations were present in varying percentage of cells regardless of fixation or hypotonicity. The configurations ranged from a highly condensed, brightly fluorescent discrete body to smaller discrete particles which were often quite widely separated. In other cells interpreted to be in very early prophase (G-2) these fluorescent particles appeared to be reaggregated into a more elongate or threadlike structure which was often reminiscent of a chromosome in metaphase.

The dispersed configuration was more frequent in cells which had been cultured than in cells which had not been cultured. In the case of lymphocytes, dispersion was seen mainly in large nuclei which had been transformed with PHA. Comparison of cultured and non-cultured lymphocytes revealed a consistently higher percentage of nuclei with dispersed morphology and this in nuclei which had a consistently larger nuclear diameter and appeared to be in some stage of transformation. In the case of pure lymphocyte preparations which had been separated from other cell types on a Ficol-
Isopaque density gradient, nearly 100 percent of the nuclei were of uniform diameter and had a condensed Y-body. These cells, when cultured with PHA showed a dramatic increase in nuclear diameter and in dispersion of the Y-body.

In other tissues examined (i.e., hair root sheath cells and buccal mucosa cells) dispersion of the Y-body was not seen. No cells in either of these tissues were observed to be undergoing mitosis.

The apparent correlation of Y-body dispersion with PHA cell transformation suggested that it was in some way related to cell division and the cell cycle. To study this possibility both short-term peripheral blood lymphocytes and long-term fibroblast cultures were harvested at periodic intervals to assess possible changes in the frequency of cells having a dispersed Y-body.

In the case of lymphocyte nuclei which were harvested at six hour intervals over a 48 hour period the frequency of dispersion showed a direct relationship to the time in culture. Mean nuclear diameter also increased directly with time in culture.

Long term fibroblast cultures were followed both at six hour intervals over a 54 hour period and at daily intervals through the logarithmic and post-logarithmic phases of cell growth. Dispersion of the Y-body showed a generally higher frequency during the logarithmic phase of growth and a decline during the post-logarithmic phase. Determination of mitotic index of the same cultures revealed an almost direct correlation of increased mitotic index with increased Y-body dispersion.
Data from both continuous terminal labeling and pulse-chase experiments involving some 2300 nuclei supported the idea that the observed morphology of the Y-body represented sequential stages of increased dispersion during the time when DNA synthesis was occurring in the nucleus.

The labeling studies also indicated that replication of the Y-body was somewhat later than the rest of the nucleus and that once DNA synthesis for the Y-body began, it occurred at a faster rate than over equivalent portions of the nucleus.

Is the Y-body Uncoiling during DNA-synthesis?

The dispersion of the Y-body would appear to represent uncoiling of the fluorescent part of the Y chromosome during the time of DNA-synthesis. The apparent dispersion, however, appears to be one of breaking up of the Y-body into a number of smaller discrete fluorescent particles rather than simply becoming more diffuse. The nature of these small particles suggest the following possible alternatives for the behavior of the Y-body:

1) The fluorescent material of the Y is uncoiling but not at a uniform rate such that the small discrete particles represent segments of material which have not completed the process.

2) The fluorescent material itself is not uncoiling, but the observed dispersion is caused by uncoiling of non-fluorescent (QM-negative) material intercalated between the fluorescent segments of the Y chromosomes.
3) The fluorescent material and intercalated non-fluorescent material are both uncoiling.

4) The dispersed material does not represent the Y-body at all but represents an aggregation of fluorescent spots in the nucleus (i.e., bright satellites) into some functional area such as the nucleolar organizer.

Of the four alternatives, the last seems the least likely, since the number of dispersed aggregations was equivalent to the number of Y chromosomes in every case and never more. The amount of dispersed material also appeared similar to the amount of fluorescent material present on the Y chromosome in metaphase. Recent evidence (Bobrow et al., 1971) indicates that the Y-body is possibly involved in a nucleolar organizer. If such is the case, and the Y-body was not actually dispersing, one would expect to find a large fluorescing Y-body in the midst of an aggregate of smaller fluorescing particles. This was not observed; the fluorescent particles making up the dispersed configurations were several times smaller than the intact condensed Y-body, and were more often uniform in size.

Concerning the first three alternatives mentioned, some additional cytological observations are pertinent. In preparations of elongate prometaphase chromosomes or late prophase chromosomes, the fluorescent part of the Y chromosome was observed to have QM negative bands interspersed in between QM positive bands. It is possible that it is the QM negative bands which are dispersing and pushing
apart the QM positive bands (alternative 2) or perhaps both types of chromatin are uncoiling and recondensing (alternative 3), but at different rates giving the illusion of beads on a string.

Two alternatives might also be proposed for the labeling activity of the Y-body:

1) The Y-body itself is labeling at a more rapid rate as it becomes more dispersed.

2) As the Y-body is becoming dispersed it is becoming associated with other DNA material which is contributing to the apparent increased rate of labeling.

The observation by Bobrow et al. that the Y-body may be involved in a nucleolar organizer suggests that the latter might be a possibility, or at least contributing to the labeling activity of the Y-body during some portion of its cycle. The possible role of the Y-body in the nucleolar organizer needs to be further investigated.

Comparison of the X-Chromatin Body and the Y-body

Since the discovery of the X-chromatin body (Barr and Bertram, 1949) in mammalian cells with two or more X chromosomes, numerous investigators have noted its variable morphology and its frequency of appearance in different tissues and under different conditions of growth. DeMars (1962) claimed the X-chromatin body was visible only in cells which were in late interphase and James (1960, 1964) claimed there was decrease in its frequency of appearance during S phase. Miles (1960) reported an inverse frequency of X-chromatin
with mitotic index, and Therkelsen and Petersen (1963) reported a decreased frequency during the logarithmic phase and an increased frequency during the post logarithmic phase of cell growth. Somewhat contradictory observations have been reported by other investigators (Schwartzacher, 1963; Klinger et al., 1967; Comings, 1967b). Schwartzacher observed the X-chromatin body in living cells over a period of 52 hours through a complete division cycle. Contrary to DeMars' observations, Schwartzacher reported the X-chromatin body to be visible throughout the cell cycle without any apparent morphological change with the exception of a single cell in which the X-chromatin body only became visible in early prophase. He did observe, however, changes in the optical properties of the X-chromatin body prior to mitosis. Autoradiographic studies by Comings and spectrophotometric studies by Klinger of DNA synthesis revealed no change in frequency of the X-chromatin body for cells which were undergoing DNA synthesis and those that were not undergoing DNA synthesis. Mittwoch (1964) reported changes in frequency of the X-chromatin body with nuclear size, a finding which was not supported by the studies of Klinger et al. or Comings. In all of the studies, however, there was observed an increase in frequency of the X-chromatin body in post-logarithmic cell growth.

A recent report by Therkelsen and Petersen (1971) on the Y-body indicated it also decreased in frequency during logarithmic growth and increased in frequency during post-logarithmic growth. In a comparison with the frequencies for the X-chromatin body, they found the two to be nearly identical.
The frequencies and relationships reported for the X-chromatin body in the cell cycle can be applied in general to the behavior of the condensed Y-body. In the conditions where no X-chromatin body is visible, the frequencies of the dispersed Y-body can be applied. Thus where an inverse relationship between frequency of appearance of the X-chromatin body and mitotic index have been reported, the same relationship was observed in the present study for the condensed Y-body, but the reverse relationship was observed for the dispersed Y-body. Similarly the observation of Therkelsen and Petersen on the frequency of the Y-body during log and post log phases of growth were confirmed with respect to the condensed Y-body but the reverse relationship was seen for the dispersed Y-body. The advantage of the present study was the use of cells having a particularly large Y-body which was visible in nearly 100 percent of the nuclei in all cultures. Therefore it must be assumed the frequencies obtained by Therkelsen and Petersen represented the condensed Y-body only, and that when highly dispersed the Y-body was either not detected by them or not scored.

A number of authors have expressed that the Y chromosome in humans may be inactive (Brown, 1966; Mittwoch, 1967a). This speculation has been based mainly on the facts that the Y chromosome, like the inactive X in females, is late-labeling, although not necessarily the last chromosome to complete replication (Takagi and Sandberg, 1968; Craig and Shaw, 1971), and that, to date, no discrete genes have been linked to the Y chromosome. Late-labeling has been
shown to be generally characteristic of heterochromatin (Lima de Faria, 1968; Brown, 1966). A number of investigators have shown that at least part of the Y chromosome is heterochromatic (Sasaki and Makino, 1963; Arrighi and Hsu, 1971). The demonstration of part of the Y chromosome as a discrete body in interphase by the recent technique of quinacrine staining would seem to add further support to the idea that the Y chromosome may be inactive, or at least may be behaving in many ways similar to X-chromatin body.

There is no special cytological marker for studying the X-chromatin body as is now represented by the bright fluorescence of the Y-body. The study of the X-chromatin body in the cell cycle has been further limited by the fact that it has only been possible to study any changes in morphology in cultured fibroblasts (the X-chromatin body is not visible amidst the condensed chromatin in lymphocytes). The variability in size of fibroblasts has produced contradictory results with regard to the frequency of the X-chromatin body in different stages of the cell cycle (Comings, 1967b; Mittwoch, 1967b; Klinger et al., 1967). In the present study the ability to detect the Y-body in all stages of the cell cycle in peripheral blood lymphocytes, in which the size of the nucleus appears to be a more accurate gauge of nuclear stage, would seem to represent a more reliable method of studying the behavior of a single chromosome than has previously been possible.

The present study opens new possibilities for studying X-chromatin and conditions affecting its frequency in nuclei (e.g. XYY)
in which both an X-chromatin body and a Y-body are present. Such studies may elucidate the behavior and role of sex chromatin in general.
V. SUMMARY

Part of the Y chromosome in humans is detectable as a brightly fluorescing body in interphase nuclei stained with quinacrine dyes. In the present study, this fluorescent body was studied in some 15 human subjects and was seen to manifest a variable morphology which appeared to be related to the stage of the nucleus in the cell cycle. The variable morphology was seen particularly in cultured fibroblasts and in peripheral blood lymphocytes undergoing blastic transformation induced by PHA, but was not observed in buccal mucosa or hair root sheath cells.

The morphology of the Y-body ranged from a highly condensed configuration in small non-transformed nuclei to increased degrees of dispersion which appeared to show direct relationship to size of the transformed nucleus. In nuclei which appeared to be in pre-prophase (G-2), the Y-body appeared to partially recondense.

Lymphocyte cultures, which were serially harvested, showed an increase in percent of nuclei with a dispersed Y-body, proportional to time in culture, and generally an increase in nuclear diameter.

Nuclei studied from serially cultured skin fibroblasts showed the highest percent of nuclei with a dispersed Y-body during the log phase of cell growth, and a decline in the frequency of dispersed morphology in the post-log phase. Percent dispersion also showed a direct relationship to mitotic index.

Continuous terminal and pulse-labeling studies of the Y-body revealed increased incorporation of tritiated thymidine with
increased dispersion. The labeling studies suggested that the observed morphological configurations were sequential, and that the Y-body while condensed generally lagged in DNA-synthesis, but that the rate of DNA-synthesis of the Y-body was more rapid than the rest of the nucleus as it become more dispersed.

The studies indicate that the different morphologies of the Y-body represent stages in the cell cycle, and suggest that portions of the Y-body may be uncoiling during the time it is actively synthesizing DNA.
VI. BIBLIOGRAPHY


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VII. APPENDIX (PLATES)
PLATE I

Comparison of Y-body size to length of the fluorescent segment on the Y chromosome.

a and b) Lymphocyte metaphase and interphase from peripheral blood of Individual V with a very short fluorescent segment on the Y chromosome, and a small Y-body.

c and d) Similar preparations from peripheral blood of Individual I with approximately 1/2 the long arm highly fluorescent, and an intermediate sized Y-body.

e and f) Lymphocyte metaphase and interphase from Individual VI with Yq+. Approximately 3/4 of the long arm is highly fluorescent. Note the large size of the Y-body.

Magnification, X 2800.
PLATE II

Series of lymphocyte interphases from Individual VI showing the proposed sequence of stages in morphology of the Y-body.

a) Metaphase spread from lymphocyte culture showing the especially long fluorescent region on the long arm of the Y chromosome.

b) Small non-transformed nucleus showing a highly condensed Y-body.

c-f) Interphases which appear to be transformed showing increased dispersion of the Y-body with increase in nuclear diameter. Note particulate nature of the dispersed fluorescence in some of the nuclei (f). The Y-body also often appeared more central or paracentral in the dispersed configurations.

g-i) Partial recondensation of the Y-body in stages which appeared to be later in the cell cycle (G-2). Note the more condensed appearance of chromatin in general and the almost metaphase-like configuration of the Y-body (i).

Magnification, X 1260.
Series of interphase nuclei of cultured skin fibroblasts from Individual VI showing similar morphological configurations of the Y-body to those seen in peripheral blood lymphocytes (Plate II). Nuclei were fixed in methanol-acetic acid (3:1) without prior hypotonic treatment.

Magnification, X 2800.
PLATE IV

Comparison of fixation and varying hypotonic treatment.

Nuclei of cultured skin fibroblasts from the same Individual with condensed, dispersed and highly dispersed configurations showing (a, c and e) fixation with HgCl₂-acetic acid (sublimate acetic) and prior isotonic treatment; (b, d and f) hypotonic treatment, 1 part BSS: 15 parts distilled H₂O, followed by fixation with methanol-acetic acid (3:1). Note the slightly swollen appearance of the latter nuclei, but no apparent affect on the morphology of the Y-body.

Magnification, X 2800.
PLATE V

Lymphocyte metaphases from XYY male showing a) metaphase spread with two Y chromosomes; b, e and g) interphase nuclei with Y-bodies at various distances apart; c) nucleus showing two dispersed Y-bodies; d) nucleus of approximately the same size showing a single large dispersed Y-body which possibly represents two Y-bodies very close together; f) nucleus showing two Y-bodies in slightly different morphological configurations.

Magnification, X 1700.
PLATE VI

Comparison of cultured and non-cultured preparations of pure lymphocytes separated from other peripheral blood cell types on a Ficol-Isopaque density gradient.

a) Non-cultured lymphocytes were small, of uniform size. The majority of nuclei had a condensed Y-body.

b) Cultured lymphocytes from same separation showed dramatic increase in size and range of nuclear diameter, and similar range in Y-body morphology as was observed in normally cultured blood.

Magnification, X 1700.
PLATE VII

Nuclei classified as being in G-1, stained with QM and labeled with tritiated thymidine for various lengths of time.

a and b) Nuclei terminally labeled for 3 minutes. Note relatively light label over one nucleus; remaining nuclei are unlabeled. Exposure time to autoradiographic film was 12 days.

c and d) Nuclei terminally labeled for 30 minutes. Note very light label over one nucleus; remaining two nuclei are unlabeled. Exposure time was 6 days.

e and f) Nuclei labeled for 13 hours. Note very heavy label over four of the nuclei. Exposure time was 2½ days.

Magnification, X 1700.
PLATE VIII

Nuclei > 20μ in diameter with a condensed Y-body from cultures labeled for different lengths of time. Label over the Y-body is relatively light and indistinguishable from label over the rest of the nucleus for all four labeling times. Exposure time to autoradiographic stripping film for 3 minute label was 12 days; for 30 minute and 5 hr label, 6 days; for 13 hr label, 2.5 days.

Magnification, X 1700.
PLATE VIII

3 Min

30 Min

5 Hr

13 Hr
PLATE IX

Nuclei with dispersed Y-body showing increased label over the Y-body with longer labeling time. Label is particularly heavy after five hours of labeling (e and f). Exposure times to autoradiographic film are identical to those given for Plate VIII.

Magnification, X 1700.
PLATE X

Nuclei with a highly dispersed Y-body showing similar labeling times to Plates VIII and IX. Note slight label over the Y-body after 3 minutes and increasingly heavier labeling over the Y-body with longer labeling times. After 13 hours distinction between label over the Y-body and label over the rest of the nucleus is less evident. Exposure times to stripping film are the same as for the previous plates.

Magnification, X 1700.
PLATE XI

Nuclei classified as G-2.

Note very light label over the nucleus after 30 minutes, and heavier label over the Y-body for the three remaining times. Exposure times are as previously described.

Magnification, X 1500.
Labeled prophases. Prophases from cultures labeled for times shorter than 80 minutes showed no label. Note increased label over Y-body with longer labeling time. Exposure time to autoradiographic film was six days for all three labeling times.

Magnification, X 1700.
PLATE XIII

Labeled metaphases from cultures labeled for 3 hrs (a and b) and 5 hrs (c and d). Note there is no label over the Y chromosome for the shorter labeling time (b), and very heavy label over the Y with the longer labeling time (d), indicating that although the Y chromosome is late labeling it is not necessarily the last chromosome to complete replication.

Magnification, X 1500.