Asynchronous Duplication of Chromosomes in Cultured Cells of Chinese Hamster

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ABSTRACT

Chromosome duplication (DNA synthesis) was studied in cultured cells of Chinese hamsters by means of autoradiography following thymidine-H^3 incorporation. The technique used was to expose an asynchronously dividing population of rapidly growing cells for a 10 minute interval to a medium with thymidine-H^3. Cells were then transferred to a medium with excess unlabeled thymidine. The population was sampled at intervals thereafter and studies made of the frequency of labeled interphases and division figures, and the patterns of labeling of specific chromosomes.

The average generation time during these experiments was about 14 hours. DNA synthesis occurred during an interval of about 6 hours and stopped 2 to 3 hours before metaphase. After metaphase the chromosomes usually begin duplication again within 5 to 6 hours.

Grain counting, to estimate the amount of tritium incorporated after a short contact with thymidine-H^3 and at intervals after transfer to a medium with excess unlabeled thymidine, indicated that the intracellular pool of labeled precursors was diluted within less than a minute so that further labeling would not be detected. The chromosomes labeled during the contact period retained their precise pattern of labeling through another duplication cycle and no turnover of DNA or loss of tritium was detectable.

Five or 6 chromosomes of the complement have segments typically late in duplication. Two of these are the X and Y chromosomes. The long arm of the X chromosome and the whole Y chromosome are duplicated in the last half of the interval of DNA synthesis. The short arm of the X chromosome in a male strain is duplicated in the first half of the interval. In another strain (female), one X chromosome had the same timing, but the other one was all duplicated in the last half of the period of DNA synthesis. The DNA in the short arms of 2 medium sized chromosomes, as well as most of the DNA in 1 or 2 of the smallest chromosomes of the complement, was replicated late.

The study has led to the hypothesis that various chromosomes or parts of chromosomes have a genetically controlled sequence in duplication which may have some functional significance.

INTRODUCTION

In many types of cells of higher organisms the synthesis of DNA (deoxyribonucleic acid) associated with the duplication of the chromosome complement requires several hours (1). However, the replication of a DNA particle (molecule)

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appears to require a much shorter time, perhaps a few minutes or less. The experiments of Meselson and Stahl (2) with bacterial DNA and of Simon (3) with DNA of mammalian cells show that at any time during the replication of DNA there exist only two types of DNA particles, parental and daughter, in detectable amounts. In these experiments DNA of a certain density, produced by incorporation of a heavy isotope or heavy base analog, is replicating in an environment containing precursors of a different density. If the individual particles did not replicate rapidly, there should exist at any time a large percentage of partially replicated particles which would attain intermediate densities, rather than the two discrete types.

The sequence of replication of the DNA particles in a chromosome or chromosome complement and the control of the sequence now becomes a pertinent question. Tritiated thymidine and high resolution autoradiography provide a means of obtaining some of the answers. The first indication that there was a regular sequence in the duplication of the chromosomes was reported by Taylor (4) for the root cells of *Crepis*. In these cells all of the chromosomes appeared to have DNA replication begining at the ends of the arms and proceeding to the centromere. Later Gall (5) reported a sequence in the macronucleus of *Euplotes* in which synthesis of DNA began at both ends of an elongated nucleus and proceeded to the middle. Lima-de-Faria (6) reported an asynchrony in the duplication of the chromosomes of a species of grasshopper in which the sex chromosome duplicated later than the remainder of the complement.

In order to get more information on the sequence in duplication, a study was begun on cells in culture. These cells have the advantage that the isotope may be delivered to the cells quickly and then depleted by changing the medium and adding carrier. This report describes the asynchrony of duplication in two strains of cells of Chinese hamster along with information on the timing and sequence of the cell division cycle and DNA synthesis. Some information on the rate of dilution of the labeled precursor pool and on retention of the incorporated thymidine-H^3 was also obtained.

Materials and Methods

Two strains of cells isolated from embryonic tissues of Chinese hamster (*Cricetulus griseus*) were supplied by Dr. George Yerganian, Children's Cancer Research Foundation, Boston. The cells were grown in Eagle's (17) medium containing 15 per cent calf serum. One strain (A1290) grew as flattened polyhedral cells, while the other (1404 ♀) grew as flattened spindle-shaped cells. Both are near diploid lines and maintained a fairly regular chromosome complement during the 2 or 3 months during which the study was made. The diploid complement typical of Chinese hamster contains 11 chromosome pairs which have been identified and numbered by Ford and Yerganian (8). Most of the cells of the strain A1290 in these cultures had 24 or 25 chromosomes (Fig. 4). Three chromosome pairs, Nos. 9 to 11, have near terminal centromeres. There are 3 of No. 9, 2 of No. 10, and 2 of No. 11 in the A1290 cells (Fig. 4). There is also 1 additional similar chromosome (indicated by ? in Fig. 4) in which the short arm, if present, is too small to see. One of the smallest chromosomes of the group Nos. 5, 6, or 7 is also present in triplicate, in most cells (Fig. 3); therefore these cells usually have $2n + 3$. There is also a heteromorphic pair of medium sized chromosomes which Yerganian (9) has identified as the X and Y chromosomes. The other strain is from a female and most of the cells now contain an extra small chromosome of the group Nos. 5, 6, or 7; therefore the chromosome formula is $2n + 1$.

The thymidine-H^3 was prepared by Schwarz Laboratories, Mount Vernon, New York. The specific activity measured by the supplier is 1.88 curies/mM. Cells were transferred to 60 mm. petri dishes containing 5 ml. of medium and a coverglass, 22 × 40 mm., and incubated at 37°C. in an atmosphere of 5 per cent CO_2 in air. After the cells had become attached to the glass and multiplied so that there were 2 to 3 × 10^5 cells per coverglass, the coverglasses were changed to dishes of fresh medium for 12 to 18 hours. The original medium in which the cells had been growing for 24 hours or less was saved for supplying isotope to the cells. To this original medium was added the thymidine-H^3. Cells on coverglasses were transferred to the labeled medium for an appropriate time, rinsed in unlabeled medium to which carrier thymidine (100 times the concentration of labeled thymidine) had been added, and finally placed in a dish of this same medium until fixed. In this way the cells were kept under conditions in which growth and metabolism would be maintained in a medium in which cells had been growing for 12 or more hours. Fluctuation in temperature during the changes was avoided in so far as possible.

Cells were fixed after rinsing briefly in Gey's salt solution (10) or after swelling for 3 to 6 minutes in a 3:7 mixture of Gey's solution and this salt solution without NaCl. Fixation was carried out by draining off excess fluid and allowing the film of fluid on the upper one-half of the coverglass to evaporate for 30 seconds to 1 minute in a stream of air at 37°C. The partially dried cells flatten and spread so that fewer of them are

lost during fixation. The coverglass with the lower half still wet was immersed in alcohol-acetic acid (3:1) for 5 to 10 minutes and then dried in the stream of warm air. The cells were usually stained by the Feulgen procedure (6 to 7 minutes hydrolysis) and dried again. The coverglasses with the cells on the outer exposed surface were cemented to slides with "euparol" (Flatters & Garnett, Ltd., Manchester, England). When these slides had dried for 2 or 3 days, stripping film (Kodak AR-10) was applied. After 1 to 3 weeks' exposure the film was developed in D-19 (5 minutes at 18°C.), rinsed, and fixed in Kodak acid fixer for 10 minutes; rinsed briefly in water and then in Kodak hypo clearing agent. After several changes of distilled water (20°C. or below) the slides were transferred to a solution of azure B bromide at pH 4.0 (40 mg. of stain and 1 gm. of potassium acid phthalate per 100 ml. of water) for 20 minutes. After a brief rinse in water the excess fluid was blotted off; the stained slides were then thoroughly dried. In this condition the slides may be stored for months or longer if necessary.

For a study of generation time and the sequence of DNA synthesis in the cell cycle a group of cultures was treated as follows. Twenty-four coverglasses with cells (strain A1290) were placed in a medium, containing 1 μc. per ml. of thymidine-H^3 (specific activity 1.88 curies/mM) for 10 minutes. Two were rinsed and fixed immediately, and the remainder were transferred to the medium with unlabeled thymidine. Preliminary experiments had indicated that no labeled division figures appeared among cells fixed 1 hour after removal from the thymidine-H^3. Colchicine (0.2 μg./ml.) was added to two cultures after 1 hour and these were fixed 1 hour later. Two additional cultures were fixed at intervals thereafter with an hour pretreatment with colchicine. Autoradiograms were prepared.

RESULTS

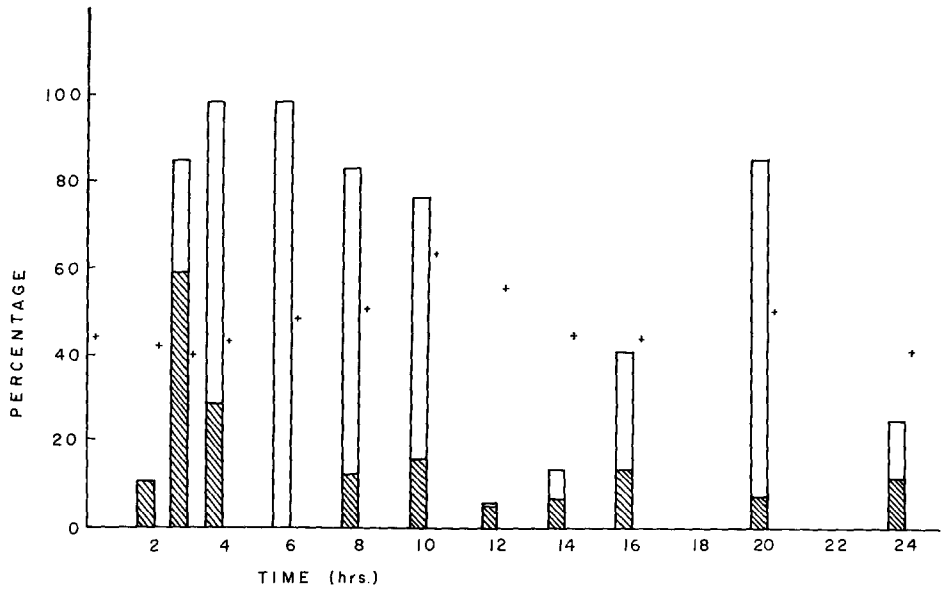
Chromosome Labeling and Cell Generation Time.— Preliminary experiments indicated that within 5 to 10 minutes cells in culture incorporate sufficient thymidine-H^3 to be readily detectable in individual chromosomes by autoradiography. When cells were transferred to an unlabeled medium the pool of labeled precursors was quickly depleted. The label was restricted to the nuclei and no labeled division figures appeared within the 1st hour.

The group of cultures prepared for determination of the generation time and the position of the DNA synthetic period in the cell cycle were examined with respect to the frequency of labeled nuclei and labeled mitotic figures (late prophases and colchicine-metaphases) in the population. By examining 200 to 250 cells at interphase and 100 to 150 dividing cells selected at random on each slide the results shown in Text-fig. 1 were obtained. In addition preliminary studies had shown that some chromosomes in the division figures were only partially labeled along their length after the short contact with thymidine-H^3 (Figs. 5 to 10). Some chromosomes appeared labeled from end to end, but in the first labeled figures to appear at division many chromosomes were completely unlabeled or labeled only in discrete regions. In examining the labeled division figures an estimate was made of the total length of the complement which was labeled. The shaded portion of the bars in Text-fig. 1 indicates the percentage of the division figures in which less than one-half of the chromosomal material was detectably labeled.

A small percentage of the labeled cells reach division within 2 hours after incorporation of the isotope in DNA. In these cells from 2 to 5 of the chromosomes contain tritium. They must represent cells which were completing DNA synthesis during the 10 minutes' contact with the thymidine-H^3. Within 3 hours 85 per cent of the division figures are labeled, but nearly two-thirds of these have less than one-half of the complement labeled. By the 4th hour 98.3 per cent of the division figures are labeled, but of these about one-third now have less than one-half the complement labeled. At the 6th hour the same percentage of division figures are labeled, but none of them has less than one-half of the complement labeled.

Thus far two characteristics are revealed. The interval between the end of DNA synthesis and metaphase varies between 2 and 3 hours, for 98 per cent of the cells arriving at division between the 3rd and 4th hour are labeled. Chromosome duplication is asynchronous, but more regions of the chromosomes are duplicating during the middle of the period of DNA synthesis than toward the end or at the beginning (Text-fig. 1).

From 8 to 10 hours following the labeling period about 20 per cent of the cells reaching division have their chromosomes labeled. The unlabeled division figures are in cells which were in the pre-DNA synthetic period of the cycle during the contact with thymidine-H^3. All of the labeled figures have unlabeled chromosomes or chromosomal regions in the complement, but only 12 to 15 per cent have less than one-half the complement labeled. By the 12th hour essentially all of the labeled cells have divided once. Some of the labeled division figures in this population are at the



TEXT-FIG. 1. Graph showing the frequency in per cent of labeled interphase nuclei (crosses) and division figures (bars) at zero time and at various intervals after a 10 minute contact period with thymidine-H^3. The shaded portion of the bars indicates the percentage of figures in which less than one-half the total length of the chromosome complement was labeled.

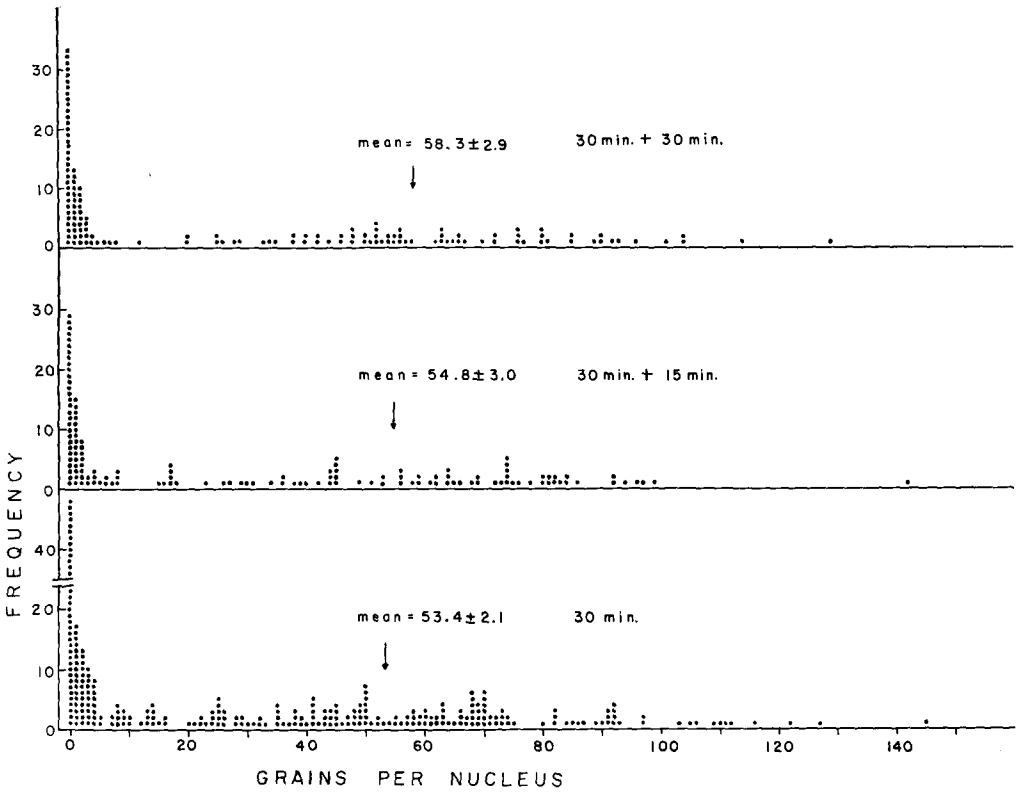
second division since being labeled, *i. e.*, only one chromatid of each labeled chromosome is now labeled.

The frequency of labeled divisions again rises to a peak at about 20 hours and thereafter falls again. A measure of the average generation time is the interval between the two peaks or about 14 hours.

In an asynchronous population of cells, the number of cells in any stage at a given moment may be taken as a measure of the average length of that stage. Therefore, after a short contact with the isotope, the percentage of labeled cells can be used to estimate the length of the period of DNA synthesis. By multiplying 0.43 (see Text-fig. 1) times 14 hours, the interval is estimated to be 6 hours. The pre-DNA synthetic interval is obtained by subtraction of 6 plus 2 to 3 from 14. This leaves 5 to 6 hours for the pre-DNA synthetic period including the mitotic stages from metaphase through telophase.

The percentage of labeled interphase nuclei (Text-fig. 1) changes during the cell cycle in the way expected of an asynchronous population of cells. The frequency drops during the first 2 to 3 hours when the population is increasing by the division of unlabeled cells. As the labeled cells divide, the frequency of label cells in the population rises to a peak 10 hours after labeling. The percentage then drops back to that equivalent to the frequency at zero time by the 14th hour, *i. e.*, after one complete cycle.

Dilution of the Pool of Labeled Precursors in Cells.—Dilution of labeled precursors of DNA could be evaluated by direct measurement of the specific activity of the acid soluble nucleotides of the cell or by the measurement of the incorporation of the labeled precursors into DNA. The latter method is feasible by means of autoradiography. A group of cultures (strain A1290) under similar conditions of growth were placed in a medium with 0.1 μc. per ml. of thymidine-H^3 (630 μc./μM) for 30 minutes. Cells on two coverglasses were fixed immediately and the remainder transferred to a medium with an excess of unlabeled thymidine. These were fixed at intervals and autoradiograms prepared. An exposure of 3 days produced a suitable number of grains for counting. The data are shown in Text-fig. 2. All of the grains over nuclei chosen at random were counted. Most of the nuclei are unlabeled and the recorded grain-count for these represents background. The mean with its standard error was calculated for the nuclei having more than 5 grains each. The differences between the grains per nucleus at 30 minutes and after an interval

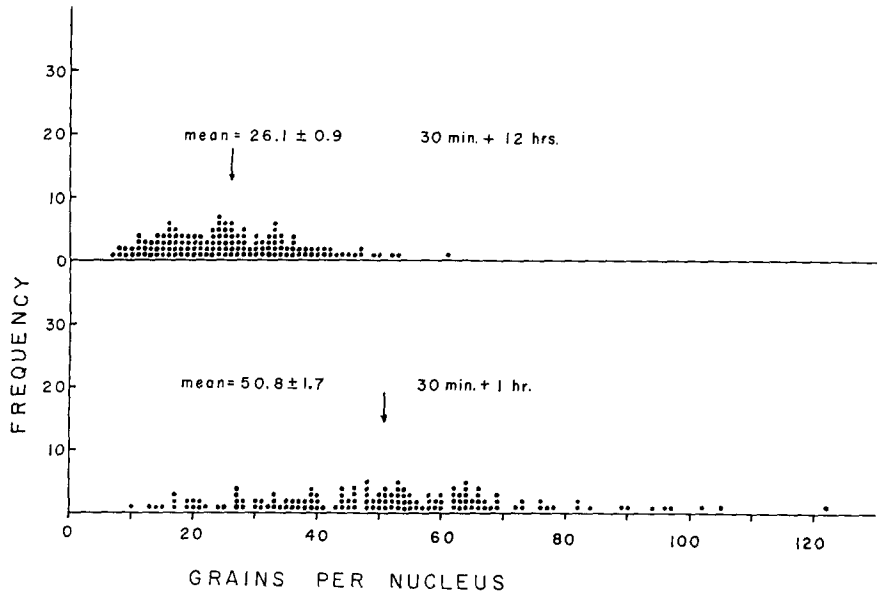


TEXT-FIG. 2. Graph showing grains per nucleus on autoradiograms of populations of cells after 30 minute contact with thymidine-H^3 (below) and 30 minutes plus 15 minutes and 30 minutes, respectively, in a medium with excess unlabeled thymidine. Mean and standard error are shown for nuclei with more than 5 grains per nucleus.

of 15 and 30 minutes are not significantly different. The dilution of the precursor pool must occur within less than a minute. An incorporation of the diluted precursor may occur, but the amount of tritium would be insufficient to be detectable in individual chromosomes. Therefore, the results on asynchrony of chromosome duplication refers to events during 10 to 11 minutes of the DNA synthetic period.

Retention of Thymidine-H^3 by the Cells.—These cells provide good material for checking the retention of the incorporated thymidine-H^3 in the presence of excess carrier. For this experiment cultures on coverglasses (stain A1290) were given 30 minutes' contact with a medium containing 0.1 μc. of thymidine-H^3 per ml. (specific activity 630 μc./μM). Two were fixed 1 hour after removal to a medium with an excess of unlabeled thymidine, two more after 6 hours, and two after 12 hours. As predicted from Text-fig. 1 no labeled cells had reached division in the first group; nearly 100 per cent of the division figures were labeled after 6 hours, and only about 1 per cent of the divisions had tritium after 12 hours. Grains per nucleus for nuclei selected at random from the two populations are shown in Text-fig. 3. Counts of 5 or less are not recorded. The mean for 30 minutes plus 1 hour in medium with unlabeled thymidine is 50.8 ± 1.7. Since no labeled nuclei have divided, this represents the average amount of isotope incorporated per nucleus during the period of contact. After 12 hours when 99 per cent of these cells have divided, there is an average of 26.1 ± 0.9 grains per nucleus.

No loss of label by exchange of the tritium or by turnover of DNA is detectable. Further evidence for this conclusion is provided by the persistence of specific patterns of labeling along the chromosomes. Precise patterns of labeling characteristically produced by contact with labeled precursors late in the DNA synthetic period (see next section) persist and show up in only one of the two chromatids of the chromosomes at the second division after labeling occurred.



TEXT-FIG. 3. Graph showing grains per interphase nucleus on autoradiograms of populations of cells in contact with thymidine-H^3 for 30 minutes. Cells were then transferred to unlabeled medium with excess unlabeled thymidine for 1 hour (below) and 12 hours (above). Mean and standard error are shown, but nuclei with 5 grains or less were not recorded.

Out of Phase Duplication of Individual Chromosomes.—Although the data in Text-fig. 1 indicate that various chromosomes or chromosome regions are duplicated at different times during the DNA synthetic period, the behavior of individual chromosomes is not revealed. The first regular behavior of identifiable chromosomes was noticed when labeled cells which reached division within 2 hours were carefully examined. In some of these only two chromosomes were detectably labeled. In the A1290 cells, these two chromosomes were a heteromorphic pair of intermediate size (Fig. 3), later identified as the X and Y chromosomes (9). The smaller of the two is the Y chromosome and in some cells it was labeled along most of the length (Fig. 7), but the X chromosome was labeled only in the long arm which sometimes showed a small secondary constriction. In some cells, arriving at division 2 hours after contact with thymidine-H^3 and with the least detectable label, only the long arm of the X chromosome distal to the constriction was labeled. The Y chromosome was labeled in some of these cells only in the short arm and the distal end of the long arm (Fig. 6). These must be the last parts of the complement duplicated. When more parts showed labeled DNA, not only the X and Y chromosome, but several of the group of smaller chromosomes, Nos. 5, 6, and 7, were labeled. In addition, the short arm of chromosome Nos. 10, 11 or perhaps both were labeled. In cells reaching division between the 2nd and 3rd hour following labeling, some tritium also appeared in the other chromosomes of the complement. In some, rather discrete regions were labeled (Fig. 5), others were more sparsely labeled along most of the length. There was a similarity in the labeling patterns of homologous chromosomes, but some non-uniformity appeared to occur.

When cells which had thymidine-H^3 available between the 7th and 8th hour before division were examined, the Y chromosome was invariably completely free of label (Fig. 8). The short arm of the X chromosome was labeled, but not the long arm. In addition several of the small chromosomes contained very little tritium. At no time during the period of DNA synthesis are all parts of all chromosomes labeled by 10 minutes' contact with the labeled precursors.

When the cells of strain 1404 ♀ were examined in the same way the two X chromosomes were found to be labeled in cells reaching division between 1½ and 3 hours after labeling. However, it was clear that the two X chromosomes were different in the timing of duplication, although they could not be distinguished morphologically.

One was labeled along the whole length while the other, as in the A1290 cells, was labeled only in the long arm (Fig. 9). The small chromosomes and the short arms of Nos. 10 and 11 also were being labeled toward the end of the duplication cycle. In cells which were labeled at the early stages of duplication, one X chromosome had tritium only in the short arm while the other had no tritium or only enough to produce 1 or 2 grains over the proximal part of the short arm (Fig. 10).

In order to estimate the period of time during which the Y chromosome was duplicating, the percentage of division figures with labeled Y chromosomes was determined at each interval following contact with thymidine-H^3. Cells reaching division between 1 and 4 hours after labeling invariably had some label in the Y chromosome. Those reaching division between the 5th and 6th hour had 11 labeled Y's to 50 unlabeled ones. Cells reaching division 7 to 8 hours after labeling occurred did not have any tritium in the Y. Therefore the Y chromosome is duplicated during the last 3 hours of the synthetic period and the long arm of the X chromosome is duplicated during the same interval.

An additional feature of the labeling pattern was observed, which is believed to be correlated with the asynchronous duplication. A small percentage of the interphase cells have grains over only small areas of the nucleus (Figs. 1 and 2). The pattern of the grains frequently corresponds in shape and size to the parts of the X and Y chromosomes known to be duplicated late. Sometimes a pattern for one or two of the small chromosomes can also be distinguished. By counting the frequency of these nuclei, a measure of the time during which these chromosomes are out of phase with the remainder may be obtained. After a 10 minute contact with thymidine, 8.2 per cent of the labeled interphases showed most of the label in what is presumed to be the X and Y chromosomes. If duplication requires 6 hours, there is a period of about 30 minutes at the end when only the Y, part of the X, parts of one or two pairs of the small chromosomes, and the short arms of chromosomes 10 and 11 are duplicating.

DISCUSSION

The retention of the tritium in the DNA with a high efficiency in the presence of an excess of carrier thymidine indicates not only the stability of the tritium atom in the thymidine residue, but the lack of any measurable turnover of the DNA during a 12 hour period. Therefore, variations in labeling along the chromosomes must be due to differences in the rate of net synthesis at the different sites. Although it is conceivable that the particles of DNA vary in their rate of replications, it is more plausible to suppose that the number of particles replicating simultaneously varies in the different chromosomal regions. All of the available evidence indicates that usually a DNA particle (molecule) replicates once and only once during a division cycle. Whether it is grouped with a whole block of particles replicating simultaneously or distributed among ones which have already replicated or will replicate later, the two daughter particles must reach a state incapable of a second replication until every particle in the complement has completed the cycle. The grouping of the simultaneously replicating particles must vary among the different chromosomes of a species and among species. In the root cells of *Bellevalia* simultaneously replicating DNA appears to be rather uniformly distributed among the chromosomes, for after a short period of contact with thymidine-H^3 all chromosomes are usually labeled from end to end (11). In root cells of *Crepis* the grouping appears to be such that those DNA subunits near the ends of the chromosome begin replication early and replication progresses toward the centromeres (4). The Chinese hamster cells described here show the most complex arrangement so far encountered.

One of the objectives in investigating the asynchronous duplication of chromosomes was to learn more about the mechanisms of control in DNA synthesis. There are apparently hormonal controls that promote or trigger growth of the type that results in cell division. These factors are probably quite indirect in their influence on DNA synthesis. In most cells there is an interval after division before DNA synthesis begins. Certain amino acid and purine analogs which stop or greatly reduce protein and RNA synthesis do not immediately stop DNA synthesis. However, under these conditions, the cells in the pre-DNA synthetic interval do not make the transition into DNA synthesis (12). Presumably, then, certain events that involve the synthesis of RNA and protein must precede the initiation of DNA synthesis. However, as shown by the sequence of duplication in the hamster cell, there are factors

controlling DNA replication which operate at the chromosomal or molecular level. After the cell is properly conditioned for DNA synthesis the process does not begin simultaneously in all of the chromosomes. The regularity in the sequences indicate a genetic control. The general similarity, but striking difference between certain homologous segments of chromosomes, is interesting in this connection. For example, the long arm of the X chromosomes, in the two strains of hamster cells examined, duplicates in the last half of the interval of DNA synthesis. In the female cells one X chromosome has its short arm duplicating early like the X chromosome in the male cells, while the other has both arms duplicating late. Therefore, the short arm of one X chromosome of the female is behaving like the long arm of the Y chromosome. Whether these arms are homologous is not completely clear, but the drawings of the sex bivalent by Matthey, Figs. 34 to 39 (13), indicate that these arms pair during meiosis with both terminal and interstitial chiasmata. If recombination does occur and the factors regulating sequences in duplication reside in each locus, then it is difficult to see how such marked differences between homologous segments could be maintained. An alternative is that the factors regulating the duplication are not part of each genetic locus, but reside in one or a few loci in each arm. Differences could then be maintained for segments as large as those detected by the autoradiographic technique.

The observation of several labeled sectors in one arm of the longer chromosomes indicates that there is no necessary sequential duplication of a chromosome as originally suggested on the basis of the situation in *Crepis* (4). In the hamster, the long arm of the X chromosome duplicates first in the proximal segment and finally in the distal portion. However, at some stages both proximal and distal segments become labeled during a 10 minute period of contact with the isotope. This indicates that there is no necessary sequential duplication of loci but that molecules of DNA may replicate simultaneously in various parts of the chromosome. In some chromosomes these simultaneously replicating molecules are located within relatively short segments, while in others this apparently is not the situation. If the molecular weight of the DNA molecules is assumed to be 8×10^6, there are perhaps between 10 and 50 thousand of these per chromosome arm. Therefore, the resolution in autoradiograms can only reveal behavior of large groups of molecules.

On the basis of asynchrony of duplication observed in the X chromosome of the grasshopper, *Melanoplus*, and in rye chromosomes, Lima-de-Faria (6) has put forward the hypothesis that heterochromatin duplicates later than euchromatin. While heterochromatin may frequently be out of phase with the other chromatin in its duplication as well as in its condensation during the mitotic cycle, the variations observed in hamster chromosomes do not fit any simple scheme. Rather there appears to be a genetic control which regulates the sequence of all of the chromosome parts. Since the two X chromosomes behave differently, one must assume that homologus regions can be either heterochromatic or euchromatic at different times, if the hypothesis relating to heterochromatin is held.

The existence of asynchrony with a highly regulated system of duplication suggests that the control is necessary for normal functioning of the cell. The duplication of a chromosome arm or a small segment early in the DNA synthetic period would have the effect of doubling that amount of genetic material in relation to unduplicated regions. Such differences could persist for a maximum of ⅓ to nearly ½ the mitotic cycle. If the chromosomes function during the period of duplication, imbalance could have a large effect on the metabolism of the cell, very much like adding an extra chromosome. Further investigation should reveal whether a certain pattern is characteristic of all of the cells of an individual or whether the pattern changes during differentiation.

Ficq and Pavan (14) and Pavan (15) have observed marked variations in the rate of incorporation of thymidine-H^3 along the giant chromosomes of *Rhynchosciara*. On the basis of these observations they have supposed that there is turnover of DNA or increase in addition to the uniform multiplication of chromonemata in certain bands or groups of bands. With the information gained from the study of asynchronous duplication in hamster cells, it becomes obvious that all of the observations on *Rhynchosciara* can also be accounted for by asynchronous duplication of the polytene chromosomes. The quantitative measurements by Rudkin and Corlette (16) in which they found the DNA had increased two times as much

in one sector of the chromosome as in another supports the idea that asynchronous duplication is involved.

Harris' (12) suggestion that incorporation of thymidine is initiated in the nucleolus in rat cells in culture is adequately explained by the asynchronous duplication of chromosomes. The chromosome with the nucleolus organizer is probably out of phase with the majority of the complement. In the small percentage of cells in which only this chromosome is duplicating the nucleolus appears to be labeled. In cells in which the nucleolus associated chromosome has not begun or has completed duplication in advance of the majority of the complement, the region of the nucleolus appears unlabeled in an otherwise labeled nucleus.

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Explanation of Plates

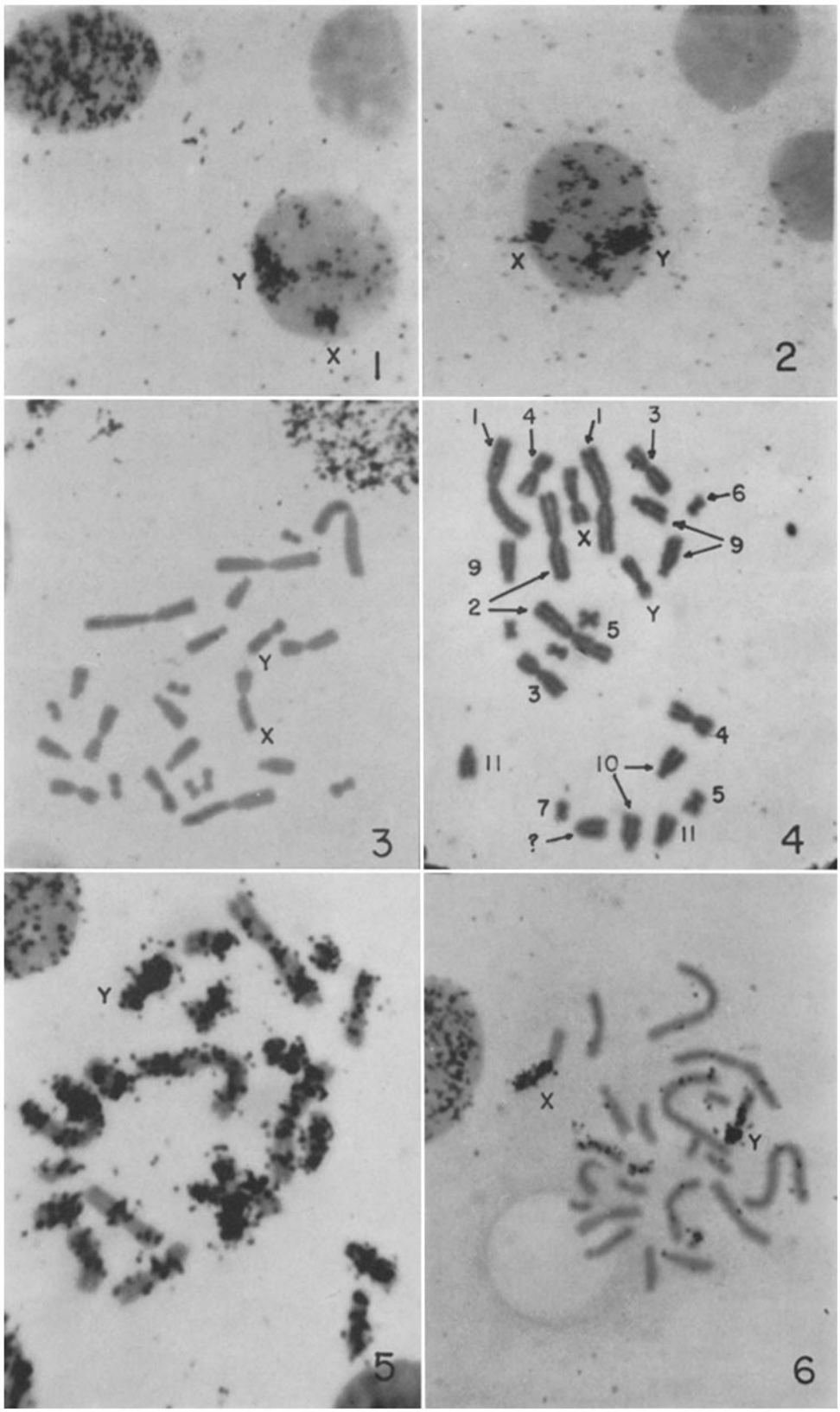
Plate 246

Figs. 1 and 2. Labeled nuclei after 10 minutes in a medium with thymidine-H^3. Most nuclei were nearly uniformly labeled as shown in the upper left of Fig. 1. The other two are labeled in discrete regions. Interpretation is given in the text. × 1580.

Figs. 3 and 4. The chromosome complement of a strain of Chinese hamster cells from a male (A1290). × 1580.

Fig. 5. Autoradiogram of chromosome complement fixed 4 hours after a 10 minute labeling period in thymidine-H^3 (strain A1290). × 1580.

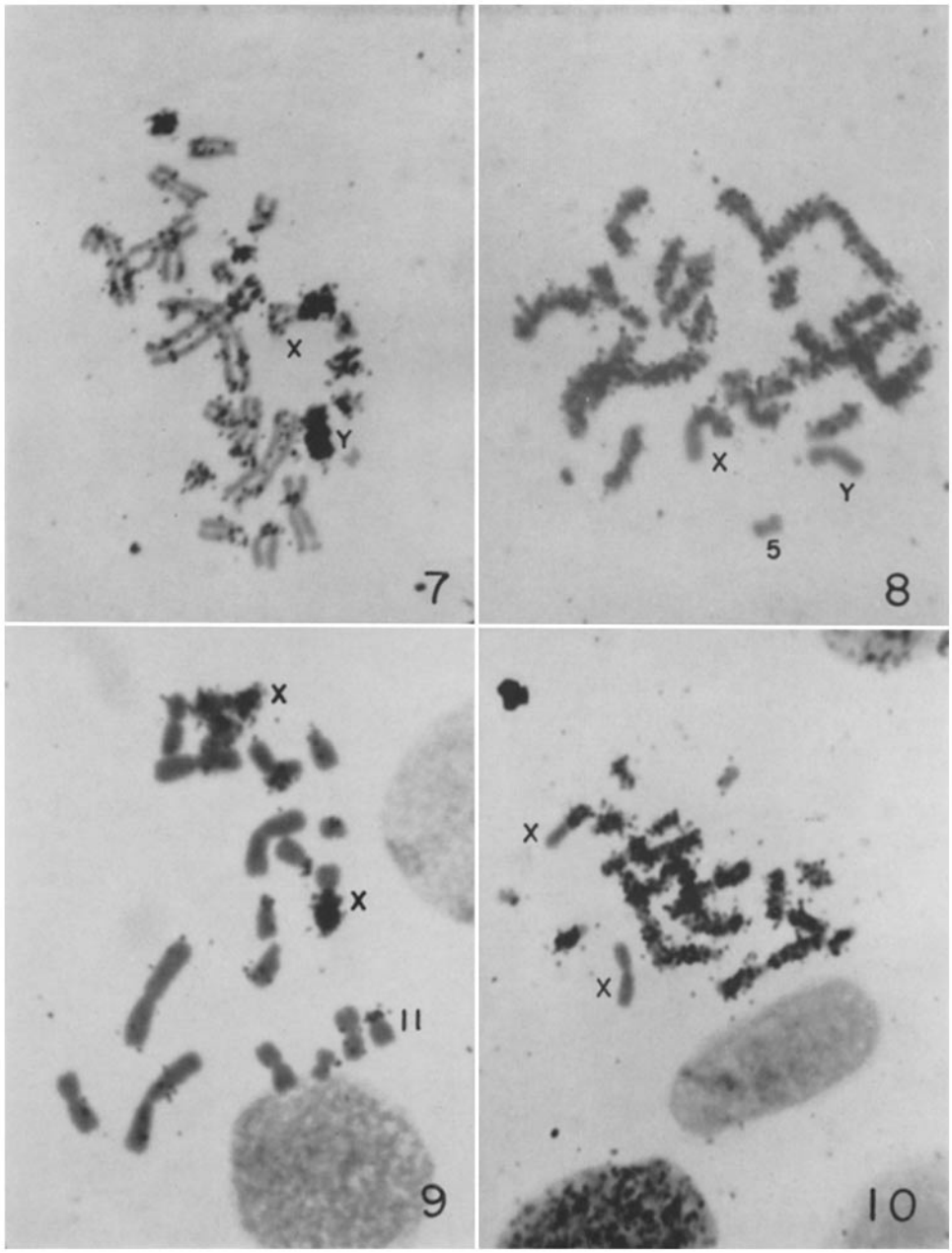
Fig. 6. Autoradiogram of chromosome complement fixed 2 hours after a 10 minute labeling period in thymidine-H^3 (strain A1290). × 1580.



(Taylor: Asynchronous duplication of chromosomes)

PLATE 247

FIGS. 7 to 10. Autoradiograms of chromosome complements of cells fixed at various intervals after a 10 minute labeling period in H^3-thymidine. × 1600. Fig. 7 shows a cell (strain A1290) labeled late in the period of DNA synthesis (fixed 2 hours after labeling). Fig. 8 shows a cell of strain A1290 which was labeled early in the period of DNA synthesis (fixed 8 hours after labeling). Fig. 9 shows a cell of strain 1404 ♀ labeled late in the interval of DNA synthesis. Fig. 10 shows a cell of the same strain labeled during the first half of the period of DNA synthesis.



(Taylor: Asynchronous duplication of chromosomes)