INCORPORATION OF TRITIUM-LABELED THYMIDINE AND LYSINE INTO CHROMOSOMES OF CULTURED HUMAN LEUKOCYTES

MAC DONALD CAVE

From the Department of Anatomy, University of Illinois College of Medicine, Chicago, Illinois. Dr. Cave's present address is the Institute of Genetics, University of Lund, Lund, Sweden

ABSTRACT

The incorporation of thymidine-H^3 and lysine-H^3 into human leukocyte chromosomes was studied in order to determine the temporal relationships between the syntheses of chromosomal deoxyribonucleic acid and chromosomal protein. The labeled compounds were incorporated into nuclei of interphase cells. Label from both precursors became apparent over the chromosomes of dividing cells. Incorporation of thymidine-H^3 occurred during a restricted period of midinterphase (S) which was preceded by a nonsynthetic period (G_1) and followed by a nonsynthetic period (G_2). Incorporation of lysine-H^3 into chromosomal protein occurred throughout interphase. Grain counts made over chromosomes of dividing cells revealed that the rate of incorporation of lysine-H^3 into chromosomal protein differed during various periods of interphase. The rate of incorporation was diminished during G_1. During early S period the rate of incorporation increased, reaching a peak in late S. The high rate continued into G_2. Thymidine-H^3 incorporated into DNA was distributed to mitotic chromosomes of daughter cells in a manner which has been referred to as a "semi-conservative segregation." No such semi-conservative mechanism was found to affect the distribution of lysine-H^3 to the mitotic chromosomes of daughter cells. Therefore, it is concluded that synthesis of chromosomal protein and its distribution to chromosomes of daughter cells are not directly influenced by synthesis and distribution of the chromosomal DNA with which the protein is associated.

The relationships between duplication of deoxyribonucleic acid (DNA) and syntheses or aggregation of various nuclear protein fractions have been studied in different tissues. The synthesis of DNA occurs during a restricted period of midinterphase referred to as S. The S period is preceded by a postmitotic G_1 (gap_1) period and followed by a premitotic G_2 (gap_2) period during which synthesis of DNA does not occur (24).

Synthesis of nuclear protein has been studied by means of microspectrophotometry (1, 6, 19, 32, 56), interferometry (30, 45), radioautography (15, 23, 51, 56), and isotope tracer techniques (12, 22, 54). The results of such experiments indicate that syntheses of various nuclear protein fractions occur during different stages of the cell cycle.

The proteins associated with the mitotic chromosomes have not been identified. It is clear, however, that chromosomes of dividing cells contain proteins (9). Although the protein of the chromosomes is not identifiable in chemical terms, it is demonstrable morphologically. Chromosomal protein may be visualized in chromosomes of mitotic cells by means of histochemical methods (28), or by radioautographic localization of la-

beled protein precursors incorporated into chromosomes (40). The latter method enables analysis of synthetic events.

A principal purpose of the present investigation has been to determine the temporal relationships between the syntheses of chromosomal DNA and chromosomal protein. Another purpose has been to ascertain how the chromosomal protein is distributed to the chromosomes of daughter cells. These problems have been approached through a study of chromosomes in dividing human leukocytes exposed to pulse treatments with tritiated thymidine (a precursor of DNA) or tritiated lysine (a precursor of protein). The appearance and distribution of the isotopic compounds in mitotic chromosomes was determined by means of radioautography. The uptake and disposition of these two precursors in the chromosomes was based on the time relative to cell division. The results indicate that incorporation of lysine-H^3 into chromosomal protein occurred throughout interphase and was not restricted to any particular portion of interphase as was incorporation of thymidine-H^3 into chromosomal DNA. Grain counts made over chromosomes of dividing cells indicate that the rate of lysine-H^3 incorporation into chromosomal protein differed during various periods of interphase. Lysine-H^3 incorporated into chromosomal protein was not distributed to the chromosomes of daughter cells in the "semi-conservative" manner described for thymidine-H^3 incorporated into chromosomal DNA.

MATERIALS AND METHODS

Culturing of Cells

These investigations were carried out on cultured leukocytes obtained from human peripheral blood.

Thirty-three blood samples were obtained from eight male and ten female individuals of normal karyotype. Samples (20 ml) were collected in two 10-ml evacuated glass tubes containing 10 units of (USP) sodium heparin. The cells in each of the two tubes containing blood taken from a given individual were maintained in separate cultures making up a culture pair. Both members of the culture pair were treated identically except that thymidine-H^3 was added to one of the cultures, and lysine-H^3 was added to the other, as detailed in a later paragraph. This permitted direct comparison of the incorporation of lysine-H^3 into chromosomal protein and the incorporation of thymidine-H^3 into chromosomal DNA.

The culturing method was a modification of one described by Moorhead et al. (34), and is based on the fact that phytohemagglutinin initiates cell division in leukocytes of normal human peripheral blood (36). From each 10-ml tube the leukocytes and supernatant plasma with some contaminating erythrocytes were collected by centrifugation at 190 *g* for 10 min. The erythrocytes were subsequently sedimented by gravity, and the supernatant plasma containing the leukocytes was inoculated into a 3-oz prescription bottle containing 8.5 ml of Puck's medium (42), 1.5 ml of calf serum, and 0.2 ml Bactophytohemagglutinin-M (Wellcome Research Laboratories, Beckenham, England). The leukocytes were cultured at 37°C. A detailed description of the culturing methods has been presented elsewhere (14).

After 50 hr of culturing, the cells were resuspended in 10 ml of Puck's medium without lysine and the incubation was resumed for an additional 10 hr. The purpose of the change of medium was to lower the lysine concentration in the medium, so that when lysine-H^3 was added to the cultures its specific activity was not excessively diluted by unlabeled lysine.

Administration of Isotope

After 60 hr of culturing, the lysine concentration in the medium was found, by analysis with automated chromatography on Dow 50 columns (39), to be approximately 0.069 mM. At this time, thymidine-H^3 (methyl labeled, 3.0 c/mmole) (Schwartz Bioresearch, Inc., Orangeburg, New York) was added to a concentration of 1 μc/ml in one member of each culture pair, and DL-lysine-4-5-H^3 dihydrochloride (3.9 c/mmole) (New England Nuclear Company, Boston) was added to a concentration of 5 μc/ml in the other member of each culture pair.

The cells were exposed to the isotope for 30 min. They were then gathered by centrifugation (45 *g*, 6 min), and were resuspended in 10 ml of fresh medium consisting of 8.5 ml of Puck's medium and 1.5 ml of calf serum, supplemented with 0.33×10^{-3} mmoles of unlabeled thymidine (100 times the concentration of thymidine-H^3) and 1.3×10^{-3} mmoles of unlabeled DL-lysine monohydrochloride (100 times the concentration of lysine-H^3).

Fixation and Mounting of Cells

At specific intervals after pulse treatment, Colcemide was added to both members of the culture pair to a final concentration of 0.004%. Thirty min after Colcemide administration, the cells were washed in 5 ml of Hanks' balanced salt solution (BSS) and were then suspended in 5 ml of hypotonic solution (20% Hanks' BSS). The hypotonic cell suspension was placed in an incubator (37°C) for 7 min. The cells were then sedimented, suspended in 4 ml of freshly prepared 3:1 methanol-acetic acid fixative, cooled to 5°–8°C, washed by changing the fixative ten

times during the next 3 to 4 hr, and finally suspended in 1.0 to 2.5 ml of fresh fixative.

The fixed cells were mounted on slides by placing one or two drops of fixative-cell suspension on a slide and igniting the fixative by the flame of an alcohol burner. This procedure provided a substantial number of well spread metaphase cells.

Radioautography and Staining

The slides were coated with Kodak AR-10 stripping film for radioautography, exposed, developed, and stained according to the method of Schmid (43). Exposure times ranged from 2 wk to 6 months.

Analysis of Data

The durations of the various periods of the intermitotic cycle were determined according to the method of Sisken (46).

After determination of the per cent of cells in mitosis and the per cent of cells labeled after exposure to lysine-H^3 or thymidine-H^3, the results were grouped according to 10-hr intervals and subjected to statistical analysis. The 0.05 level was selected as the criterion for significance in all statistical tests. A simple one-way analysis of variance was carried out to test the null hypothesis. The percentages were transformed by arc sine transformation which tends to normalize the distribution. Cochran's test was used to test the hypothesis of homogeneity of variance. In the event of a statistically significant F ratio, Duncan's multiple range test employing the tables constructed by Harter was used to determine which of the 15 possible differences between means were responsible.

Grain counts were made over the chromosomes of mitotic cells. All slides on which counts were made were coated with stripping film, exposed, developed, and stained at the same time, in order to make the grain counts strictly comparable. Mitoses in which the chromosomes were well spread with minimum overlapping were selected for grain counting.

RESULTS

Identification of Labeled Components

Lysine-H^3 and thymidine-H^3 were localized in the nuclei of interphase cells and in the chromosomes of mitotic cells. Cells which had been prepared in the described manner, air dried, and mounted in media of various refractive indices were examined with the Baker-Smith interference microscope. Nuclei of interphase cells and mitotic chromosomes were found to have been freed of cytoplasm. Cytoplasm was apparent in cells which were simply taken from culture, smeared on slides, and air dried without hypotonic treatment or fixation. Thus, labeled cytoplasmic proteins were removed during the preparative procedures. Proteins soluble in the fixative solution accounted for the somewhat greater background label apparent on slides prepared from cell cultures treated with lysine-H^3.

Cells which were exposed to thymidine-H^3 were treated with deoxyribonuclease (Sigma Chemical Company, St. Louis, 1 time crystallized) 0.5 mg/ml in 0.002 M Tris (hydroxymethyl) aminomethane, 0.005 M $CaCl_2$, and 0.45 M $MgCl_2$ at pH 7.3 for 60 min at 37°C. The thymidine-H^3 label was completely removed by deoxyribonuclease extraction. Buffer controls showed that the extraction was attributable to the enzyme. The results indicate that tritium in cells labeled with thymidine-H^3 was present in a DNA component of the nucleus.

Cells exposed to lysine-H^3 were extracted for 30 min with 5.0% trichloroacetic acid at 90°C. The nuclear-chromosomal label in the lysine-H^3–labeled cells was resistant to the hot trichloroacetic acid extraction, suggesting that the label was present in a nonnucleic acid component of the chromosomes, probably protein.

Uptake of Thymidine-H^3 and Lysine-H^3 into Chromosomes

The fact that, in both the lysine-H^3 and thymidine-H^3 experiments, there was no significant variation in the per cent of cells undergoing mitosis during the 60 hr after initiation of isotope treatment indicates that mitotic synchrony had not been induced in the cells by the culturing procedures. Cells in mitosis accounted for between 0.2 and 2.1% of the total number of cells in cultures exposed to thymidine-H^3, and between 0.3 and 2.4% of the total number of cells in cultures exposed to lysine-H^3.

At the time of pulse treatment, an estimated 98 to 99% of the cells were in interphase. The first cells to approach mitosis were in the terminal portion of interphase at the time of isotope administration. As time after pulse treatment proceeded until one divison cycle had been completed, the cells entering mitosis were in progressively earlier periods of interphase at the time of isotope administration.

In Fig. 1, the per cent of mitotic cells showing chromosomal label after treatment with thymidine-H^3 and lysine-H^3 has been plotted against time after initiation of pulse treatment. From the

data on thymidine-H^3 uptake, the average generation time for the dividing cells was calculated as 17.7 hr. The average durations of G_1, S, and G_2 were 4.6, 9.6, and 3.5 hr, respectively (Fig. 1 *a*). The per cent of labeled mitoses showed three successive increases, indicative of cells undergoing mitosis for the first, second, and third time after thymidine-H^3 incorporation.

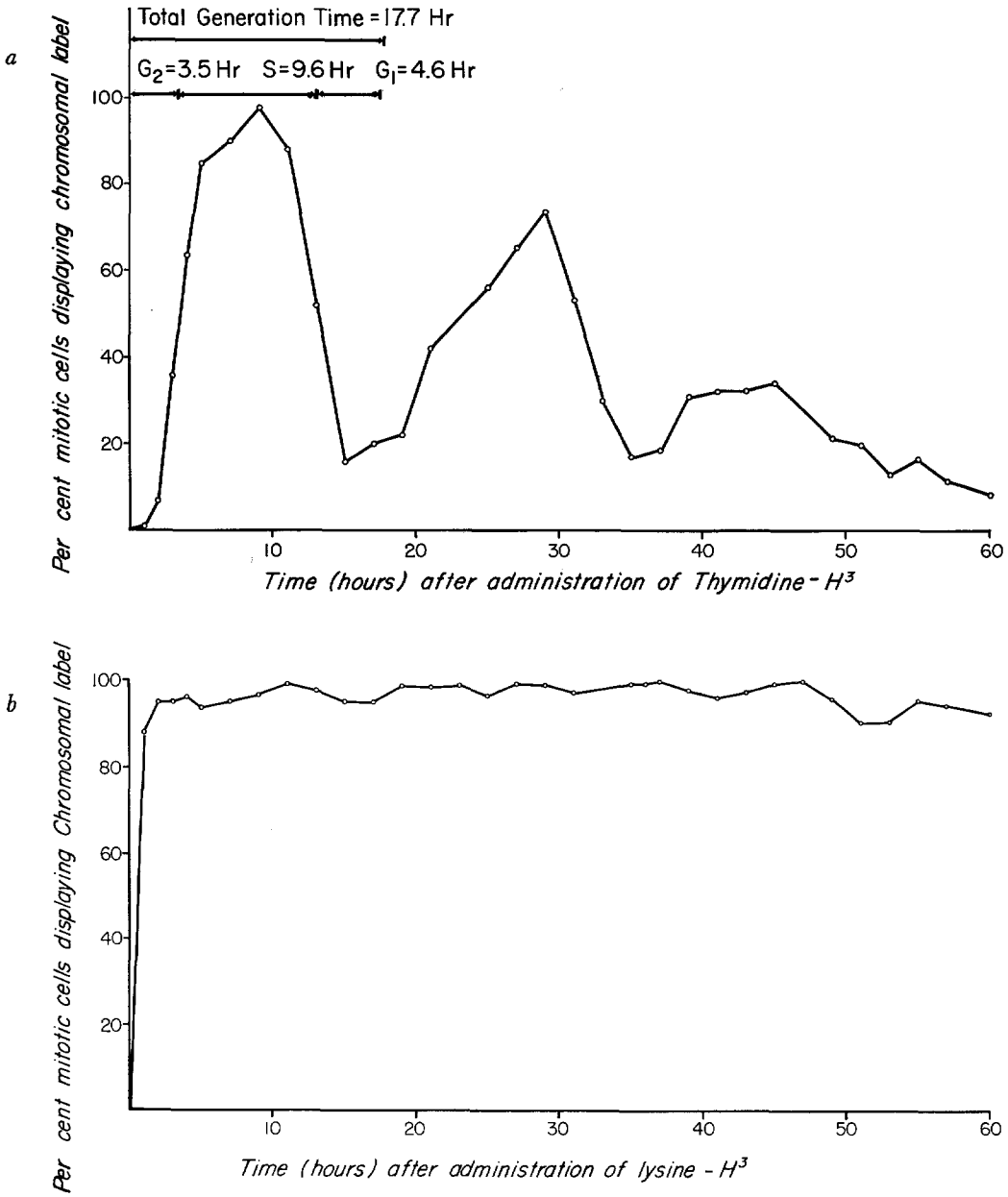


FIGURE 1 Graph showing the per cent of mitotic cells displaying chromosomal label after isotope administration. For each time interval all mitotic cells on a single slide were counted.

a, After administration of thymidine-H^3. An average 581 mitotic cells from a given culture were counted for each time interval. *b*, After administration of lysine-H^3. An average 332 mitotic cells from a given culture were counted for each time interval.

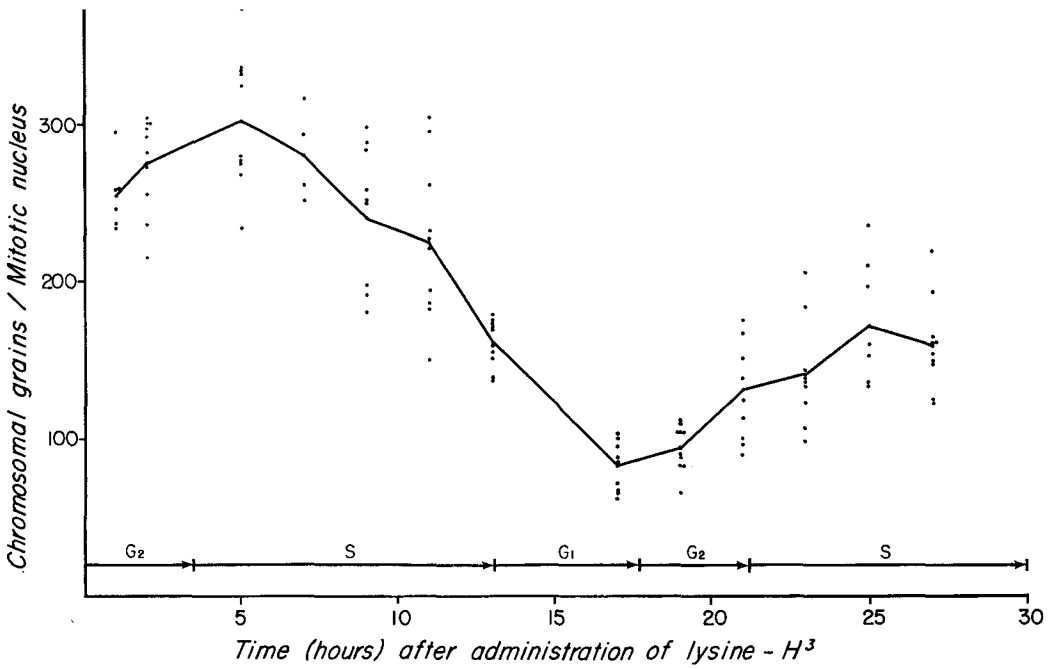


FIGURE 2 Incorporation of lysine-H^3 into chromosomes. The average number of chromosomal grains/mitotic nucleus is indicated by the solid line. The grain counts were corrected for background. Background is the difference in grain count between the area occupied by the mitotic figure (exclusive of the chromosomes) and an adjacent area of the same size.

The per cent of mitotic nuclei showing chromosomal label after pulse treatment with lysine-H^3 contrasted with that of the thymidine-H^3 experiments (Fig. 1 *b*). In the thymidine-H^3 experiments, the per cent of mitotic cells displaying chromosomal label varied throughout the 60 hr after initiation of pulse treatment (Fig. 1 *a*). This variation was due to the fact that thymidine-H^3 was only incorporated into chromosomal DNA during the S period of interphase. The S period was preceded and followed, respectively, by the G_1 and G_2 periods during which thymidine-H^3 was not incorporated. Such variation did not occur in cells labeled with lysine-H^3 (Fig. 1 *b*). The data indicate that incorporation of lysine-H^3 into chromosomal protein occurs throughout interphase and is not restricted to the period of interphase during which thymidine-H^3 is incorporated into DNA.

Grain Counts Made over Chromosomes Labeled with Lysine-H^3

In order to determine whether the rate of lysine-H^3 incorporation into chromosomal protein was the same throughout interphase, grain counts were made over chromosomes of mitotic cells which had incorporated lysine-H^3 (Fig. 2). Counts were made on slides which were exposed to stripping film for 2 months.

During the period when most of the cells which passed through mitosis were in G_1 at the time of isotope treatment, the average number of chromosomal grains/mitotic nucleus was diminished. During the period when most of the cells which passed through mitosis were in S at the time of isotope treatment, the average chromosomal grain count increased and reached a peak as cells which were in a late portion of S phase at the time of isotope administration entered mitosis. The high grain count characteristic of S continued as cells most of which were in G_2 at the time of isotope administration entered mitosis.

The data indicate that the rate of lysine-H^3 incorporation into chromosomal protein differs during various periods of interphase. The rate is diminished during G_1. During S period the rate increases, reaching a peak in late S. The high rate continues into G_2.

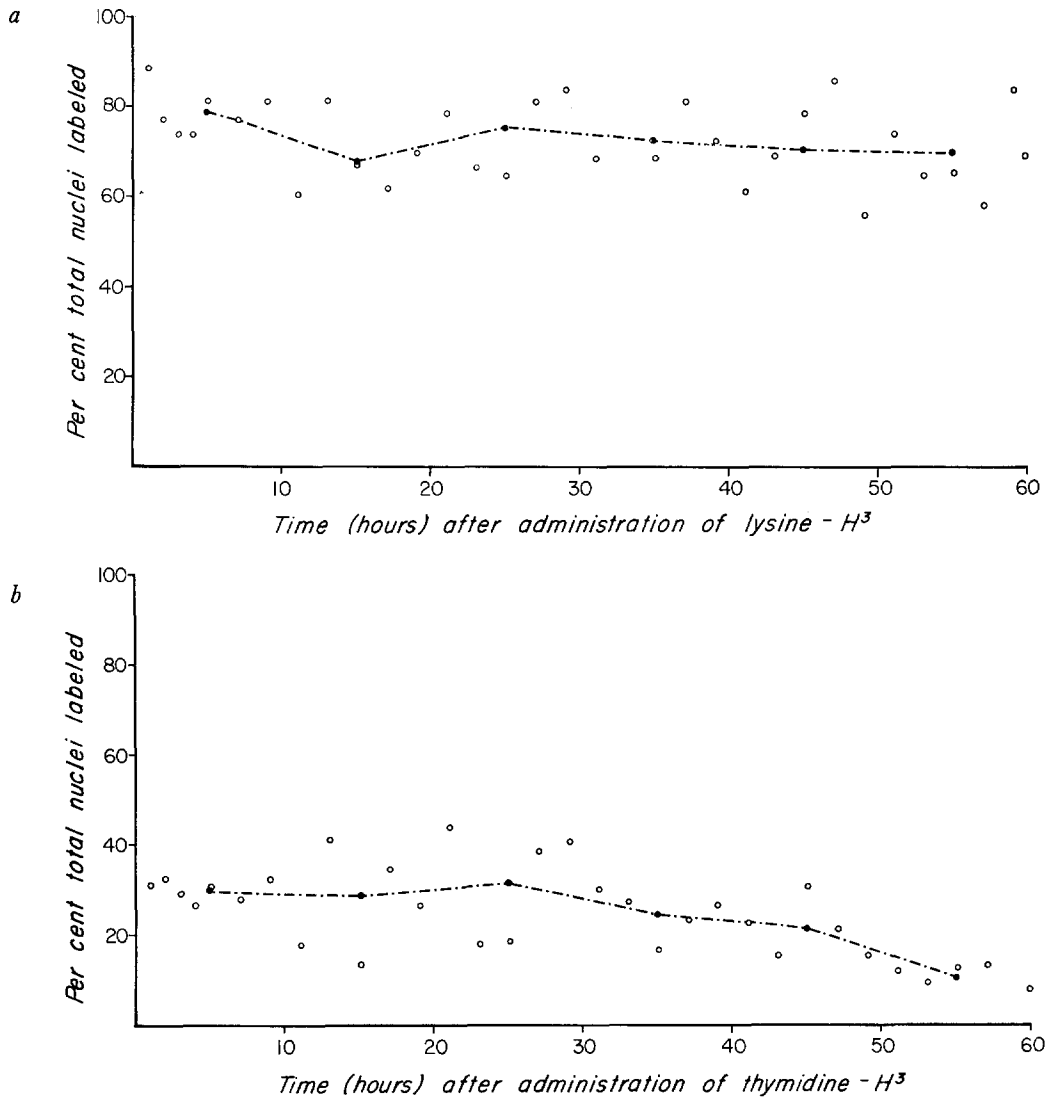


FIGURE 3 Graph showing the per cent of total cells displaying nuclear label after isotope administration. For each time interval approximately 1,000 cells from a given culture were counted.
a, After lysine-H^3 administration. *b*, After thymidine-H^3 administration.

If metabolic turnover of labeled protein were to account for the variations in grain count throughout interphase, then the average number of chromosomal grains/mitotic nucleus would be expected to decrease after pulse treatment and not to increase a second time, since labeled lysine lost from protein turnover would be diluted by excess unlabeled lysine in the media and not reincorporated.

However, a second increase in the average number of chromosomal grains/mitotic nucleus did occur between 17 and 25 hr after initiation of pulse treatment. This increase was caused by a second division of cells which were, at the time of isotope administration, in a period of the cell division cycle during which lysine-H^3 was more actively incorporated into chromosomal protein (G_2 and late S).

The data indicate that the lysine-H^3 incorporated into chromosomal protein was rather stable. The fact that the average number of chromosomal grains in the second peak was approxi-

mately half that of the first indicates that most of the lysine-H^3 present in the chromosomes at the time of the first division after isotope incorporation was passed to the chromosomes of daughter cells. The chromosomes of each daughter cell received half the amount of lysine-H^3 found in the chromosomes of the parental cell.

Per cent of Cells Incorporating Isotope into the Nucleus

Nuclei of interphase and mitotic cells were examined in order to determine the proportion of the total cells incorporating isotope during the pulse exposure. The per cent of nuclei labeled with thymidine-H^3 and the per cent of nuclei labeled with lysine-H^3 were plotted against time after initiation of pulse treatment (Fig. 3).

In cells pulse-labeled with thymidine-H^3 the variation in the per cent of cells showing nuclear label during 50 hr after initiation of pulse treatment was not statistically significant (Fig. 3 *a*). The significant decrease in the per cent of cells displaying labeled nuclei observed between 51 and 60 hr after initiation of pulse treatment is probably accounted for by an inhibition of cell growth effected by thymidine-H^3. This effect has been described by Painter et al. (37) and by Grisham (21).

In an asynchronous population of cells, the percentage of cells in any stage at a given moment provides an estimate of the average period of time spent in that stage. During 50 hr after initiation of pulse treatment, when there was no significant variation in the proportion of cells showing nuclear label, an average 27.4% of the cells were labeled. Calculations which take into consideration the age distribution of the cells (27) show that 27% is a lower proportion than expected for cells with a generation time of 17.7 hr and an S period of 9.6 hr. The data indicate that not all of the cells in a culture were capable of DNA synthesis. This was confirmed by other experiments which showed that 30 to 40% of the cell population did not undergo DNA duplication and subsequent division. Granulocytic leukocytes which do not show a mitotic response to phytohemagglutinin probably account for the nonduplicating population.

In cells pulse-labeled with lysine-H^3, the variation in the per cent of cells showing nuclear label was not statistically significant during 60 hr after initiation of pulse treatment (Fig. 3 *b*). An average 73.5% of the cells incorporated lysine-H^3 into nuclei. This was somewhat less than that which would be expected on the basis that cells incorporated lysine-H^3 throughout interphase. Perhaps nuclei of cells which did not undergo DNA synthesis and associated division did not incorporate lysine-H^3. This would explain why less than 100% of the cells displayed nuclear label.

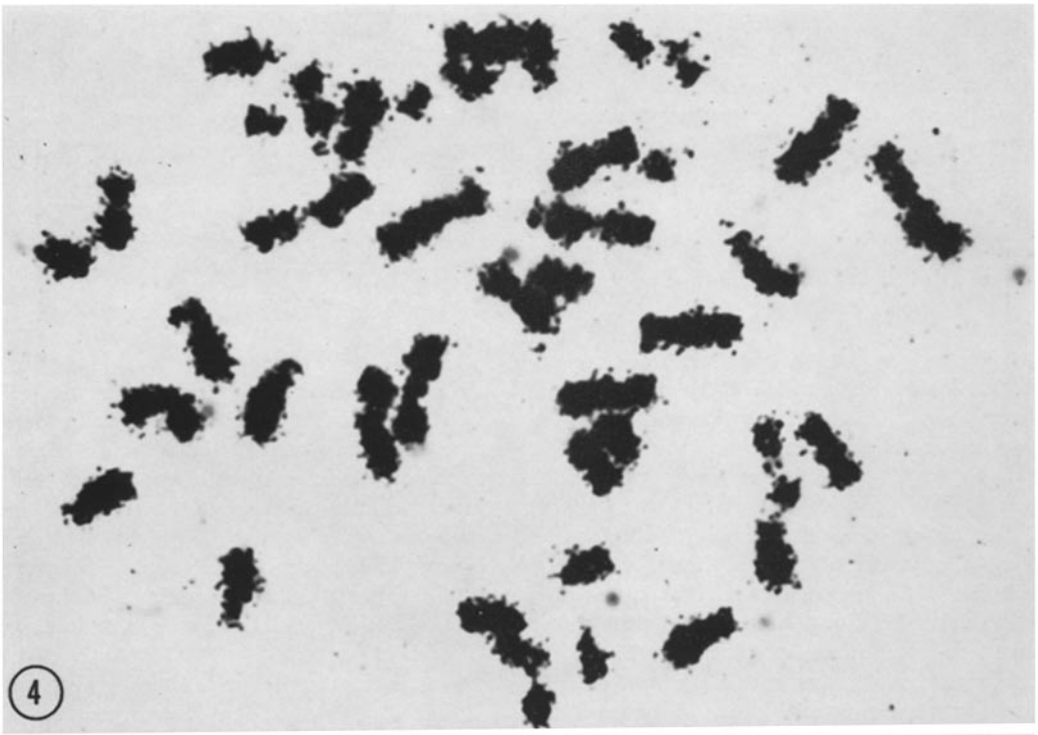
Distribution of Label to Chromosomes of Daughter Cells

In order to determine whether or not thymidine-H^3 and lysine-H^3 are distributed to the chromosomes of daughter cells in a similar fashion, the chromatid distribution of the labeled compounds was followed during 60 hr after initiation of pulse treatment.

During 19 hr after initiation of pulse treatment with thymidine-H^3, the silver grains over chromosomes of all labeled mitotic cells were localized over both chromatids (Fig. 4). Such a label distribution is characteristic of cells undergoing their first division after thymidine-H^3 incorporation. In chromosomes of cells which were in the first division after lysine-H^3 incorporation (between 0 and 19 hr after initiation of pulse treatment), the label was distributed along the arms of both chromatids (Fig. 7). The chromosomal label was very intense at this time.

Between 21 and 35 hr after initiation of thymidine-H^3 treatment, the silver grains over the chromosomes of most labeled mitotic cells were localized over one chromatid. The other chromatid had no silver grains associated with it (Fig. 5). Cells labeled in this manner were undergoing the second division after isotope incorporation. In chromosomes of cells most of which were in the second division after lysine-H^3 incorporation (between 21 and 35 hr after isotope treatment), the label was still distributed along the arms of both chromatids (Fig. 8). The chromosomal label was, in general, somewhat diminished.

Silver grains over labeled mitotic cells between 37 and 60 hr after initiation of thymidine-H^3 exposure were distributed in a characteristic manner, with one chromatid in about half of the chromosomes of a cell being covered with silver grains. The remainder of the chromosomes were unlabeled (Fig. 6). Such a label distribution indicates that these cells were undergoing their third division after isotope incorporation. In chromosomes of cells most of which were in the third



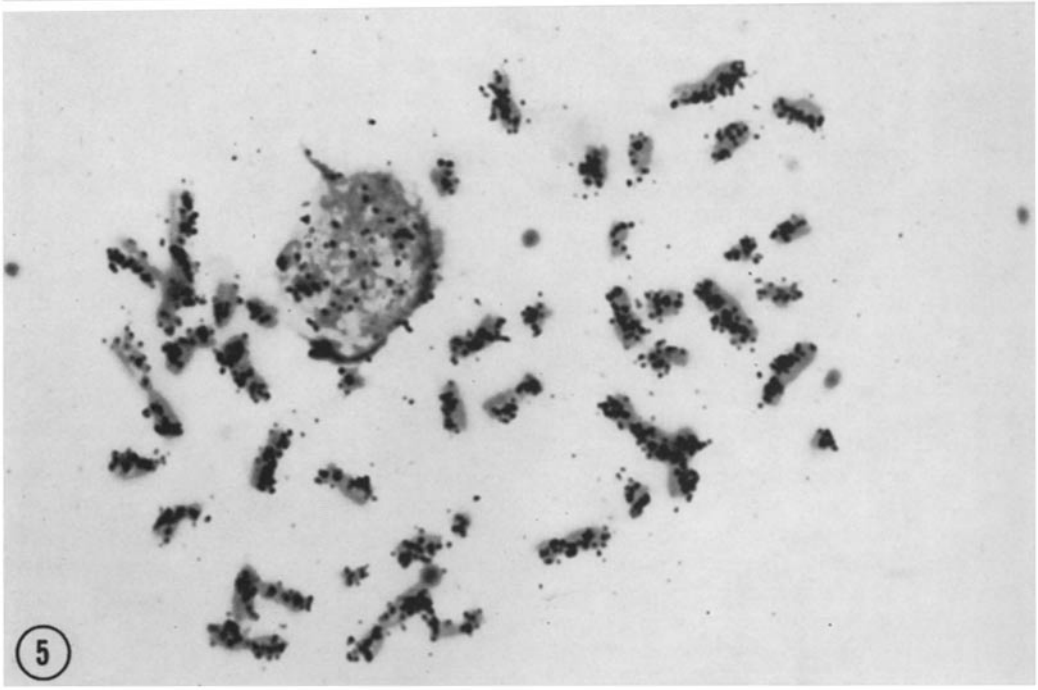
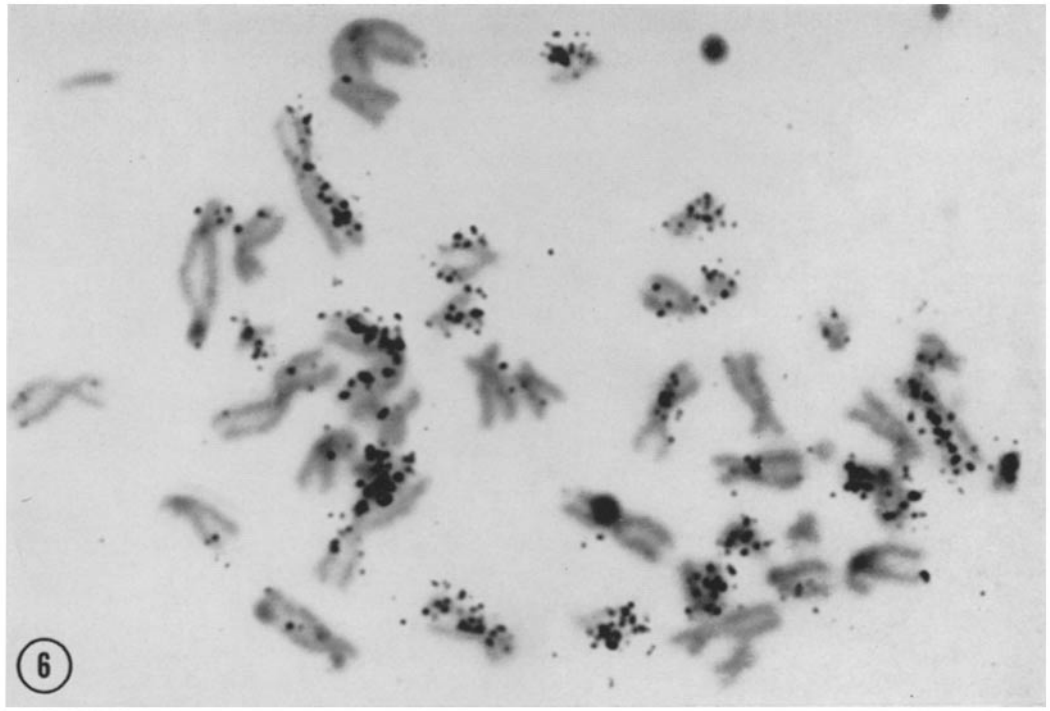


FIGURE 4 Radioautograph of chromosomes from cell labeled with thymidine-H^3 5 hr prior to fixation. Label is distributed over both chromatids of each chromosome. Radioautograph exposed 6 wk. × 1200.

FIGURE 5 Radioautograph of chromosomes from cell labeled with thymidine-H^3 21 hr prior to fixation. Label is distributed over one chromatid of each chromosome. Radioautograph exposed 6 wk. × 1200.



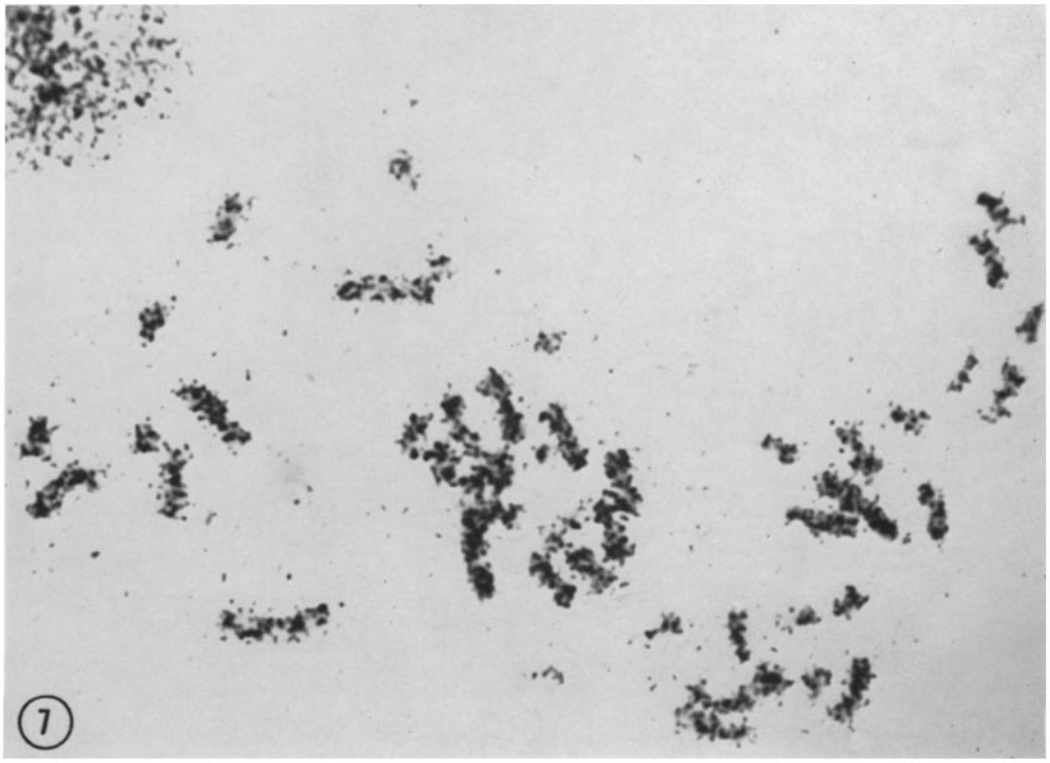
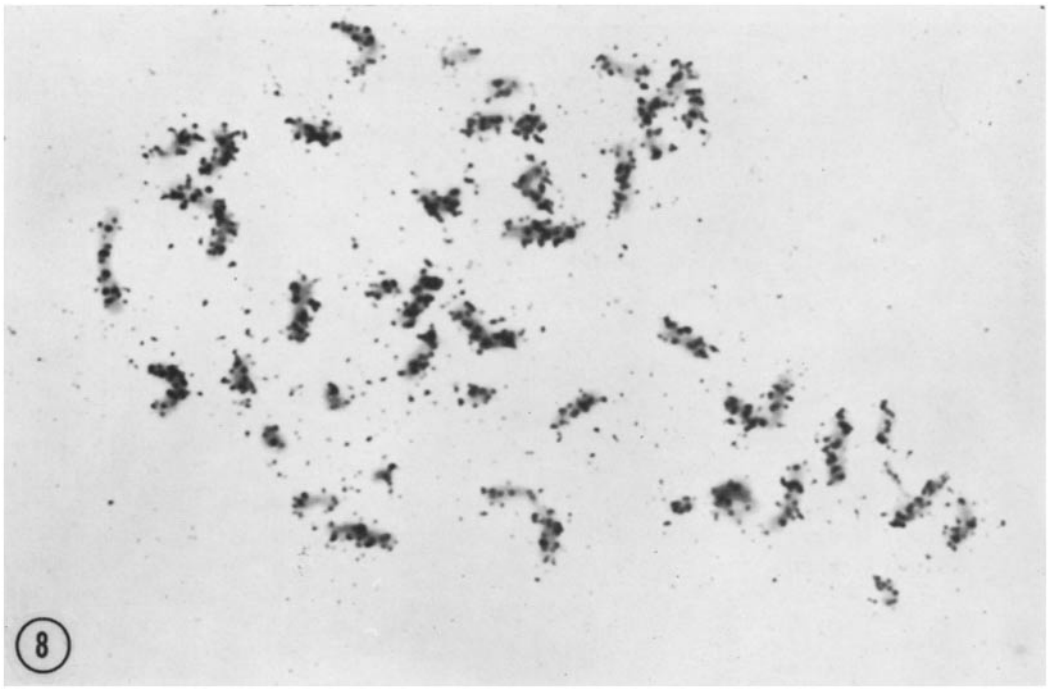


FIGURE 6 Radioautograph of chromosomes from cell labeled with thymidine-H^3 41 hr prior to fixation. About half the chromosomes of the cell are labeled, and the label is over but one chromatid of each of these. Radioautograph exposed 6 wk. × 1500.

FIGURE 7 Radioautograph of chromosomes from cell labeled with lysine-H^3 5 hr prior to fixation. Label is distributed over both chromatids of each chromosome. Radioautograph exposed 5 months. × 1100.



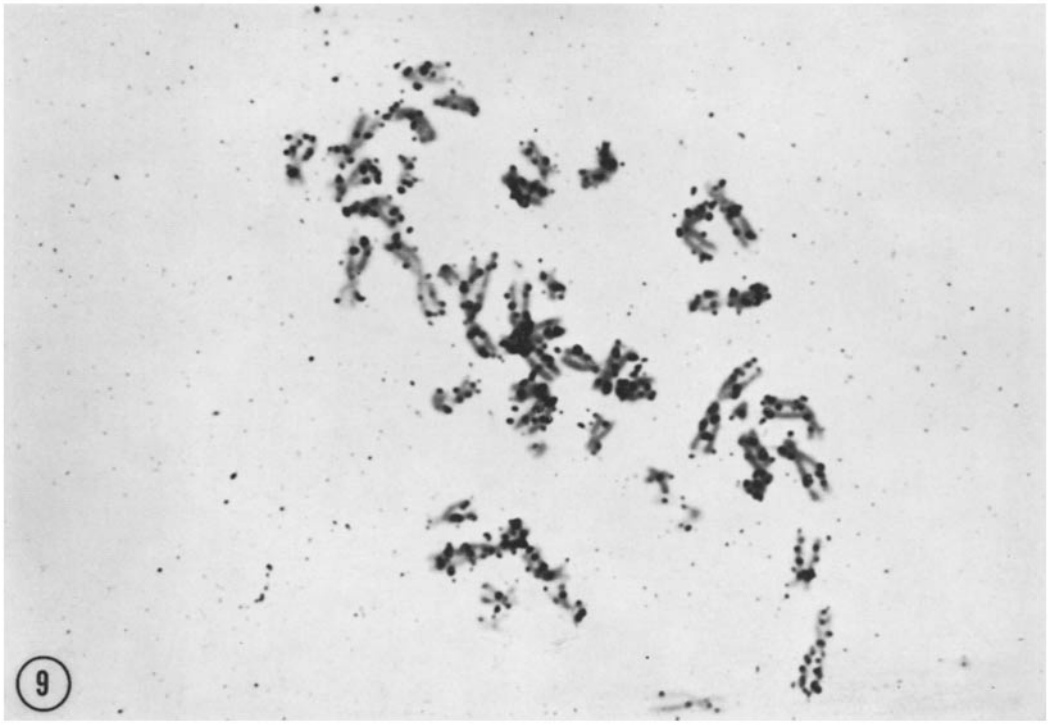


FIGURE 8 Radioautograph of chromosomes from cell labeled with lysine-H^3 27 hr prior to fixation. Label is distributed over both chromatids of each chromosome. Radioautograph exposed 5 months. × 1200.

FIGURE 9 Radioautograph of chromosomes from cell labeled with lysine-H^3 47 hr prior to fixation. Label is distributed over both chromatids of each chromosome. Radioautograph exposed 5 months. × 1200.

division after lysine-H^3 incorporation (between 37 and 60 hr after initiation of pulse treatment), the label was distributed along the arms of both chromatids (Fig. 9). The intensity of the chromosomal label was further diminished at this time.

Some mitotic cells were found between 37 and 60 hr after thymidine-H^3 administration which showed the label distribution characteristic of the second division after isotope incorporation. The occurrence of such cells during this time indicated that differences in generation time existed among individual cells.

DISCUSSION

In the present experiments, thymidine-H^3 label was sensitive to treatment with deoxyribonuclease. This is in agreement with other studies in which the chromosomal nucleic acid component labeled with thymidine-H^3 has been identified as DNA (3, 4, 18, 25, 44).

The isotope in lysine-H^3–labeled nuclei was resistant to hot trichloroacetic acid hydrolysis, suggesting that the isotope was present in a protein component of the nucleus and chromosomes. The fact that the intensity of the label was only slightly diminished by extraction with weak acid solutions (0.25 N HCl, 1.0 N HCl, and 0.01 N HCl) indicates that most of the labeled nuclear-chromosomal protein did not have the solubility characteristics of histone. Various histone fractions have a high lysine content (11, 26, 35) and are known to incorporate lysine-C^{14} (8, 10). Therefore, it may be concluded that most of the histone has been removed from the cells by the preparative procedures, or has been rendered HCl unextractable due to changes in solubility characteristics of histones denatured by fixation. That the histone has been removed by the preparation procedures is indicated by the fact that the cell nuclei give only a very faint alkaline fast-green test for histone. These observations are in agreement with those of Busch and Hsu (9), who found that most of the histone in mitotic chromosomes is removed by fixatives containing 30 to 50% acetic acid. Most of the histone in nuclei of onion root meristem cells was removed by 3:1 alcohol–acetic acid fixation (15).

Proteins which have been found to be associated with the mitotic chromosomes include the histones (2), the acidic nuclear proteins (28, 31), various enzyme systems (9, 55), and other proteins which have not yet been identified (9). Most of the histone is removed from the chromosomes or rendered HCl unextractable by the preparative procedures. It is also likely that other chromosomal proteins are so affected by these procedures. Therefore, the lysine-H^3 labeling observed in the present experiments is defined in terms of its chromosomal location and its resistance to extraction by the preparative procedures. Within this context it is referred to as chromosomal protein.

The generation time of the dividing cells and the average durations of the various periods of the intermitotic cycle were very similar to those reported for other mammalian cells (33, 41, 50). The average durations of G_2 and S phase were in good agreement with those found in cultured leukocytes from individuals afflicted with Down's syndrome (13). The duration of G_2 was approximately equal to that reported for cultured leukocytes by German (20). The durations of S and G_1 in cultured human leukocytes were found by Kikuchi and Sandberg (29), and Prescott and Bender (40), to be somewhat longer than those observed in the present experiments. The differences can be accounted for by the fact that the latter authors measured the lengths of the initial G_1, S, and G_2 periods which freshly drawn leukocytes underwent in culture. The cells studied in the present experiments would have undergone at least one intermitotic DNA synthesis cycle in vitro at the time of pulse treatment.

The present observations reveal that lysine-H^3 was incorporated into chromosomal protein throughout interphase, and that incorporation was not restricted to a particular period of interphase as was the incorporation of thymidine-H^3 into chromosomal DNA. Furthermore, the grain count data indicate that during the G_1 period the incorporation of lysine-H^3 into chromosomal protein was diminished. It increased during S, reaching a peak during late S. The high rate of lysine-H^3 incorporation continued into G_2.

The observations do not rule out the possibility that a particular nuclear or chromosomal protein fraction (i.e., histone) was synthesized during a limited portion of interphase.

Microspectrophotometric data indicate that the histone and basic nuclear protein fractions stainable with alkaline fast green (2) or Sakaguchi's reagent increase simultaneously with DNA content (1, 6, 19, 32, 56). The increase, however, does not necessarily imply histone synthesis, but might

be accounted for by the complexing of previously synthesized histone with DNA of the nucleus, the DNA-histone complex rendering the previously synthesized histone stainable (7).

Biochemical determinations on the nuclei of regenerating liver cells have indicated that synthesis of histone and of other basic nucleoproteins occurred prior to the onset of DNA synthesis (12, 16, 22, 54). However, this increase in nuclear protein may have been due to replacement of protein lost by catabolic breakdown of nuclear proteins. Such breakdown occurred after partial hepatectomy (54). Therefore, the nuclear protein increase observed in regenerating liver experiments is likely to be the result of a pathological process rather than a process that occurs in normal tissues.

Interferometric measurements made on nuclei of various cell types have revealed that the total amount of nuclear protein did not increase after cell divison until a certain waiting period had elapsed. Thereafter, the increase in total nuclear protein and the increase in DNA proceeded simultaneously (30, 45). Those results are similar to these of the present investigations, indicating a diminished rate of nuclear protein synthesis during G_1.

The work of De (15) and Woodard et al. (56) on plant cell nuclei indicated that synthesis of nonhistone proteins of the nucleus occurs throughout interphase. Radioautographic evidence indicated that the rate of nonhistone synthesis increased during late interphase and that the rate of histone synthesis was greatest at the time of DNA synthesis. Since the proteins of the chromosomes have not been characterized in chemical terms, it was not possible to extend these observations to chromosomal protein.

Several investigators have studied the incorporation of isotopically labeled amino acids into giant chromosomes in the salivary glands of various insect larvae (17, 38, 47–49). The cells were fixed in acetic acid-containing fixatives. The labeled amino acids were incorporated into chromosomal protein, and the label was distributed uniformly along the entire length of the chromosomes. Label was most intense in regions of the chromosome in which DNA synthesis was occurring (17). The labeled chromosomal protein did not have the solubility characteristics of histone. These data support the findings of the present experiments.

Chromosomes of several types of plant cells have been labeled with various protein precursors (23, 51, 52). The results show that maximum incorporation occurred during DNA synthesis.

The distribution of thymidine-H^3 to chromatids of daughter cells was originally described in plant cells by Taylor et al. (53). Observations on cultured peripheral blood cells (5, 41) revealed similar events. The results indicated that thymidine-H^3 which is incorporated into DNA of the chromosome is part of a physical entity that remains intact during successive replications and nuclear divisions. The basic chromosome is made up of two functionally complementary DNA-containing units which undergo replication to form four units. The units segregate in such a way that each daughter cell receives an old and a new unit.

The results pertaining to the distribution of lysine-H^3 to the chromosomes of daughter cells are in agreement with the findings of Prescott and Bender (40). Those workers found that incorporation of tritiated amino acids into the mitotic chromosomes of cultured Chinese hamster fibroblasts could be demonstrated by means of radioautography. Cells which were in the first and second mitoses after labeling showed label distributed over both chromatids of each chromosome.

If the labeled chromosomal protein is intimately associated with the individual DNA strands, one would expect the two materials to segregate in a similar fashion. Moreover, the syntheses of these two chromosomal components might be expected to occur simultaneously or show definite temporal relationships to one another. The results of the present experiments show that the chromosomal DNA and chromosomal protein do not segregate in like fashion, nor do their syntheses occur simultaneously. Therefore, the results suggest that the synthesis of the chromosomal protein studied and its distribution to chromosomes of daughter cells are not directly influenced by the synthesis and segregation of the DNA strands of the chromosome with which the protein is associated.

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