Short Title:

DNA SYNTHESIS IN MAMMALIAN SEX CHROMOSOMES

DNA SYNTHESIS IN MAMMALIAN SEX CHROMOSOMES: ESPECIALLY THE SEX CHROMOSOMES IN BOVINE CULTURED LEUKOCYTES

by

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ABSTRACT

DNA SYNTHESIS IN MAMMALIAN SEX CHROMOSOMES: ESPECIALLY THE SEX CHROMOSOMES IN BOVINE CULTURED LEUKOCYTES

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Chromosomal DNA synthesis was studied autoradiographically in bovine leukocytes by pulse labeling cultures with H^3TdR and harvesting samples at intervals thereafter. The cell cycle consists of G1, S and G2 periods of 2, 8 and 2 hours, respectively. The sex chromosomes in both sexes begin replication at the beginning of the S period along with the autosomes. The early replicating X chromosome and the two longest autosomes in the female replicate at a constant rate throughout the S period. The late replicating X chromosome of the female and the Y chromosome of the male begin replication at relatively low rates and constantly accelerate throughtout the S period until they are replicating at relatively high rates in the late S period. The experimental results were related to the topics of heterochromatin-euchromatin and gene action and dosage compensation in mammalian sex chromosomes.

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CLAIM OF ORIGINAL RESEARCH

This is to certify that the study reported in this thesis constitutes the original work and contributes to the knowledge in the field of mammalian cytogenetics.

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I. INTRODUCTION

Deoxyribonucleic acid (DNA) was shown by Avery et al. (1944) to be the material by which inherited characteristics are passed on from one generation to the next. The molecular structure of DNA and its mode of replication was discovered by Watson and Crick (1953a,b) and was supported by studies by Wilkins et al. (1953). The basic structure of DNA is that of a double helix of nucleotides complexed with basic proteins, called histones, which may be arranged in the grooves of the helix and bound with the phosphate groups of the DNA. The organization of DNA in chromosomes is still not clearly understood, although several models have been postulated by various authors (Freese, 1958; Taylor, 1963; De, 1964; Uhl, 1965a,b; Du Praw, 1965).

The sequence of DNA bases is maintained and passed on from mother cells to daughter cells by the mechanism of DNA synthesis, or replication. The relatively weak hydrogen bonds holding the complementary base pairs together in a DNA double helix are severed in a particular physiological environment of the cell separating the two strands of the double helix. In the presence of DNA polymerase, DNA precursors, cations and an energy source, nucleotides pair with the exposed bases in a specific manner so that each single-strand of the original helix acts as a template for the assembly of the new strand of two filial double-stranded helices (Jehle, 1963, 1965; Watson and Crick, 1953b).

When is DNA synthesized in the life cycle of a cell? This question was answered by Howard and Pelc (1953). Using Vicia faba root tip cells, autoradiography and labeled DNA precursors, they observed three distinct periods in interphase. Initially, there is a period when no DNA is synthesized, called the G1 period. At the end of this period the cell moves into the interval of DNA replication or S period. This is followed by another period of no DNA synthesis, called the G2 period. After the completion of these three periods of interphase the cell divides and the daughter cells enter G1 period.

Other relevant questions about DNA replication concern the problems of whether or not all the chromosomes of a complement replicate together throughout the S period, the rates of DNA replication in individual chromosomes, and the differences in replication between euchromatin and heterochromatin.

Using autoradiography and labeled DNA precursors, Lima-de-Faria (1959) observed that in cells from male grasshoppers (Melanoplus) the sex chromosomes, which form a heterochromatic block during meiotic prophase, replicate their DNA later than the euchromatic autosomes during the pre-meiotic S period. Asynchrony of replication between heterochromatin and euchromatin was also reported by the same author in Secale, in which each chromosome of the complement has a darkly-stained heterochromatic segment around the kinetochore.

Taylor (1960), using essentially the same technique as Lima-de-Faria (1959), studied the pattern of DNA synthesis in Chinese hamster cells in culture. The results of his experiments clearly indicated that the long arm of the X chromosome and the entire Y chromosome of the male complement replicate much of their DNA in the late S period. In the female, one entire X chromosome and one-half of the other X chromosome replicate during the late S period. Parts of some of the autosomes in both male and female complements were also found to replicate late in the S period.

German (1962a,b) investigated the patterns of DNA replication in cultured lymphocytes from the human male and female. The significant finding from this experiment was that one X chromosome in the human female, but not in the male, was late in replicating its DNA compared with the rest of the complement.

These significant observations led the way for a series of studies on the patterns of DNA replication in normal and abnormal cells from various animals and man. The results of these DNA replication studies, along with information on the cell cycle analyses, are summarized in the Appendix. The significant conclusions derived from the above-mentioned and related studies led to the following conclusions:

(1) One X chromosome in a mammalian female complement replicates most of its DNA late in the S period and at rates greater than the rest of the complement.

(2) The Y chromosome in mammalian males also replicates most of its DNA late in the S period at relatively high rates.

(3) The sex-chromatin mass observed in interphase nuclei of mammalian females formed by the heterochromatinization of one X chromosome (Barr and Bertram,1949; Barr,1951)replicates asynchronously with the rest of the complement (Atkins, 1962).

(4) In diploid complements with various numbers of X chromosomes, the number of late-replicating X chromosomes is equal to the maximum number of sex-chromatin masses present in interphase nuclei and one less than the total number of X chromosomes present in the complement (Barr and Carr, 1960; Ford et al., 1959a; Jacobs and Strong, 1959; Ford et al., 1959b; Jacobs et al., 1959; Ferguson-Smith et al., 1960).

(5) Either the maternal or the paternal X chromosome of a mammalian female complement is late replicating in different cells of the same animal (Mukherjee and Sinha, 1964; Evans et al., 1965).

(6) Generally speaking, in embryos before implantation, the late replicating portions of the sex chromosomes tend to replicate synchronously with the autosomes and do not show the late replicating pattern. This coincides with the absence of the Barr body in embryos before implantation. (Kinsey, 1967; Hill and Yunis, 1967).

(7) There are two types of spermatogonial cells; one shows the typical somatic replication pattern of the sex chromosomes, and the

other type shows sex chromosomes that replicate synchronously or earlier than the autosomes and do not show the late replicating pattern. The replication pattern of the second type of spermatogonia is also found in spermatocytes (Ghosal, McGill Ph.D. Thesis, 1968).

The above conclusions derived from the replication studies and associated cytological observations support the concept of differentiation of the sex chromosomes in mammalian cells. Other cytological observations also support this concept of X chromosome differentiation. For example, in prophase cells from mammalian females one X chromosome is heteropyknotic and more darkly-stained than the rest of the complement (Ohno and Hauschka, 1960; Ohno and Makino, 1961). Furthermore, a modified method of fixation (Saksela and Moorhead, 1962) gives metaphase figures in which one X chromosome in the female and the Y chromosome in the male are negatively heteropyknotic with a fuzzy, diffuse morphology.

The cytological observations on the differentiation of the X chromosomes, reviewed above, correlate with the genetic observation that mammalian females, heterozygous for an X-linked gene, have a mosaic phenotype. Such observations on the peculiarities of the expression of X-linked genes in the mouse formed the basis of an hypothesis proposed by Lyon (1962). This hypothesis on gene action and dosage compensation of X chromosomes postulates that:

(1) The single X chromosome which forms the sex-chromatin mass in interphase cells of mammalian females is genetically inactive.

(2) This inactive X chromosome may be of maternal or paternal origin in different cells of the same individual.

(3) The inactivation of one X chromosome in each cell occurs randomly during early embryogenesis, and in descendant cells the same X chromosome remains inactivated.

Pooling all the available information, it became apparent that the chromosome which forms the sex chromatin mass in interphase, the heteropyknotic chromosome observed at prophase, and the X chromosome that replicates at a higher rate than other chromosomes in the late S period, is the same X chromosome that undergoes differentiation in early development and becomes genetically inactive.

Although the Lyon hypothesis has been widely accepted in genetic circles, several objections have been levied against it that are derived from cytological and genetic observations. Ohno (1963) observed that the heterochromatic chromosome is not always functionless in female mice. In 30 per cent of the metaphse figures observed, the nucleolus organizer was associated with the heterochromatic X chromosome while in 70 per cent of the figures it was associated with the euchromatic X chromosome. Gruneberg (1967b) based on his genetic observations recently proposed a rival hypothesis which states that both X chromosomes of the female are equally active genetically but that the activity of each chromosome is only half that of the X chromosome of the male in order to get dosage compensation between the sexes. The Gruneberg hypothesis however, lacks an explanation for the many cytological observations and DNA replication studies that support the concept of X chromosome differentiation in the female.

This dispute on the matter of gene action and dosage compensation of the X chromosome in mammals has not been clarified to date. An attempt was made in the Discussion of this thesis to present a compromise hypothesis suggested by the observations made in the present study.

Replication studies may have further genetic significance other than in the area of X chromosome differentiation and gene action. Taylor (1960) pointed out that the segments of chromosomes that replicate in the early S period are then present in a tetraploid state for a longer time than segments that replicate in the later parts of the S period. If the genes function during the S period then this asynchony of replication of chromosomes may have an effect on the metabolism of the cell. Since studies on chromosome replication suggest that individual complements follow a regulated pattern of duplication, it would be of value to find out when specific genes located on specific segments of chromosomes are replicated in the S period. To date, studies on the patterns of chromosome replication have dealt mainly with the chromosomes or chromosome segments that replicate in the late S period, especially in the very terminal stages.

Information concerning the patterns of replication of chromosomes or chromosome segments during the early parts of the S period is inconclusive and contradictory. In his study on replication of DNA in HeLa S3 cells, Painter (1961) has indicated that the onset of DNA synthesis is simultaneous in all chromosomes. This conclusion has been supported by Stubblefield and Mueller (1962). However, German (1962a,b) and Kikuchi and Sandberg (1964) observed that, in cultured leukocytes from normal human males and females, DNA synthesis did not begin simultaneously in all chromosomes. In his autoradiographic studies on chromosome duplication in cultured cells from the Chinese hamster, Taylor (1960) observed that the long arm of the X chromosome and the entire Y chromosome in the male, and in the female the long arm of one X chromosome and all of the other X chromosomes duplicate only during the last half of the DNA synthetic period. Hsu (1964) studied the initiation of DNA synthesis in the late replicating X and Y chromosomes of a diploid cell strain from the Chinese hamster male. By inhibition of DNA synthesis with the application of 5-fluorodeoxyuridine (5-FUdR) to the culture and then relieving the inhibition with the addition of tritiated thymidine (H^3TdR), he obtained evidence which suggests that the entire late replicating X chromosome and long arm of the other X chromosome begin DNA synthesis later than the rest of the complement. Petersen (1964), following a comparable labeling technique, independently identified one chromosome in the normal human female complement which was late in initiating its DNA synthesis. This chromosome was presumed to be in the late-replicating X chromosome. The above and the following reports suggest that the late replicating X chromosome is correspondingly late in beginning replication: Graves (1967) in the kangaroo and wallaby; Schneider and Reike (1967) in the opossum; Chang et al. (1965), and Bianchi and De Bianchi (1966a) in the rat; Huang (1968) in Rattus (mastomys) natalensis; and in man, Morishima et al. (1962), Gilbert et al. (1962), German (1964), Hsu and Lockhart (1964), Cave (1966), Comings (1967b), Ockey et al. (1966), Priest et al. (1967), Sofuni and Sandberg (1967, 1968), and Takagi and Sandberg (1968a,b).

Using the Chinese hamster cells and FUdR technique, Stubblefield (1965) made repeated autoradiographs of the distribution of H^3TdR in the chromosomes of a single cell labeled at the initiation of the S period. After the separate autoradiographs were combined into a single photograph, it became clear that the Y chromosome and the long arm of the X chromosome do indeed incorporate a significant amount of isotope during the early S period and, therefore, are not late in beginning DNA replication. Making use of a continuous labeling technique and a normal human female complement (Mukherjee and Sinha, 1965), a human complement with an X/iso-X sex chromosome constitution (Mukherjee et al., 1966), and the cow complement in which the sex chromosomes are the only submetacentric chromosomes (Mukherjee et al., 1967), it was shown that in the mammalian female the late replicating X chromosome and the other X chromosome simultaneously begin replication, even before some autosomes. Other reports that give evidence to support this position are: Evans _et al._ (1965) in the mouse; Bianchi (1966b) in the rat; Brown _et al._ (1966) in the dog; Gartler and Burt (1964), Mukherjee _et al._ (1968), and Wright (this thesis) in cattle; and Bianchi and De Bianchi (1965) in man.

The replication pattern of the mammalian Y chromosome as reported in previous studies appears to be similar to that of the late replicating X chromosome in the female. According to these reports the Y chromosome replicates in the late S period at higher rates than the rest of the complement. Initiation of DNA replication in the Y chromosome has not been studied as extensively as its termination. Late initiation of DNA synthesis on the Y chromosome was reported by: Schneider and Reike (1967) in the opossum; Taylor (1960), and Hsu (1964) in the Chinese hamster; Gartler and Burt (1964) in cattle; Bianchi and De Bianchi (1965), Sofuni and Sandberg (1967) and Takagi and Sandberg (1968a,b) in man. On the other hand, evidence that initiation of replication of the Y chromosome occurs in the early S period was reported by Kikuchi and Sandberg (1964) and Cave (1966).

Relatively little has been reported on the rates of DNA replication in individual chromosomes of a complement during the entire S period. Takagi and Sandberg (1968a) using human lymphocyte cultures reported that the rate of DNA synthesis for the entire complement gradually accelerates to a peak of activity in the mid S period and rapidly decreases near the end of the S period. However, their data also suggested that some individual pairs of chromosomes maintain a constant rate of replication during most of the S period. A constant rate of replication throughout the S period has also been reported for Ehrlich ascites tumor cells *in vivo* (Edwards *et al.*, 1960) and of L-cells *in vitro* (Stanners and Till, 1960; Dendy and Cleaver, 1965).

Before studying the rates of replication throughout the S period in a given complement or in its individual chromosomes, the cell cycle must be analyzed. One reason for such an analysis is that it must be known exactly when in the S period the metaphase figures obtained from various harvests after pulse had incorporated H^3TdR. Furthermore, variation does exist in the average duration of different periods of the cell cycle among various mammalian complements, or even among the same cell strains in different cultural conditions, so the extent of this variation must be known in the particular cell population studied.

II. PURPOSE OF THE EXPERIMENTS

Experiments were designed to provide further information in relation to the studies discussed in the preceeding pages. The aims of the experiments are the following:

1. To determine the duration of the cell cycle and its various phases in cultured bovine lymphocytes.

2. To determine the relative rates of DNA synthesis throughout the S period in the X chromosomes and the two longest autosomes of the bovine female complement and in the sex chromosomes of the bovine male complement.

3. To determine the patterns of initiation of DNA replication in the sex chromosomes of bovine female and male complements and in the two longest autosomes of the female complement.

III. MATERIALS AND METHODS

1. The Bovine Chromosome Complement:

Lymphocytes from peripheral blood of a bovine male and female (*Bos taurus*) were used for the present experiment. The advantage of using the bovine complement (2n=60) for this experiment is that the submetacentric sex chromosomes can be easily distinguished from the acrocentric autosomes. The X chromosome is as long as the longest pair of autosomes, whereas the Y chromosome is comparable in size to the twenty-third and twenty-fifth autosomal pairs (Sasaki and Makino, 1962); (see Plates 1 and 6).

2. The Lymphocyte Culture:

Normally, mammalian peripheral lymphocytes do not synthesize DNA as demonstrated with human lymphocytes by Bond *et al*. (1958). According to this estimate no more than 0.06 per cent of the circulating white cell population synthesize DNA. *In vitro*, lymphocytes do not synthesize DNA unless phytohemagglutinin, or some other mitogen, is added to the culture (Nowell, 1960). Upon addition of such a mitogen, lymphocytes undergo a transformation and begin DNA synthesis. Phytohemagglutinin is an extract of the red kidney bean, *Phaseolus vulgaris*. Its mitotic stimulating fraction can be separated from the fraction which accounts for red cell agglutination (reported in Bach *et al*., 1964). The mitogenic activity was found to be due to a particular protein associated with a mucopolysaccharide, which was analysed by physical methods and found to be homogeneous. (Rigas and Johnson, 1964).

When PHA is added to a lymphocyte culture (Moorhead *et al.*, 1960), two major events occur in the first 24 hours. First, the granulocytes disintegrate, thereby reducing the heterogeneity of the cell population in the culture (Yoffey *et al.*, 1965). Secondly, PHA stimulates the small, medium and large lymphocytes to undergo a parallel series of transformations from cells with relatively dense chromatin and little cytoplasm to large blast-like cells with enlarged nuclei containing less dense, more granular chromatin and with more cytoplasm that becomes highly vacuolated. The classification of lymphocytes by size may not be particularly meaningful since the variation probably reflects different stages of maturation or differentiation of the same cell type (Stewart and Ingram, 1967). Fifty to seventy-five per cent of the lymphocytes are irreversibly stimulated to undergo transformation in the first five hours of culturing in the presence of PHA. This transformation is considered irreversible after five to ten hours of culture because even if PHA is then removed the cells will continue to go through the later stages of transformation (Kay, 1967). It has been reported that shortly after exposure to PHA the nuclear histones of the lymphocytes are acetylated (Pogo, Allfrey and Mirsky, 1966) and nuclear proteins are phosphorylated (Kleinsmith, Allfrey and Mirsky, 1966) before induction of RNA synthesis occurs.

In the second 24-hour interval, the few remaining granulocytes disintegrate (Pegrum and Barry, 1967), transformation of lymphocytes proceeds at a rapid rate (Yoffey *et al.*, 1965), and DNA synthesis begins, as indicated by the incorporation of H^3TdR (Yoffey et al., 1965; Sinha and Mukherjee, unpublished data, 1965). During this interval the mitotic index was estimated to be about 1.3 (Yoffey et al., 1965).

Seventy-two hours after the addition of PHA very few untransformed cells with small, dark nuclei are found in the culture (Bach et al., 1964). The transformed cells at this time are larger and more vacuolated than at 48 hours. The mitotic index in this population is approximately 4 (Astaldi et al., 1967).

3. The Method of Lymphocyte Culture:

The method of lymphocyte culture used in the present experiments was based on the standard method of Moorhead et al. (1960). The blood samples were drawn from the cattle using a heparinized syringe and transferred to 15 ml. centrifuge tubes. After centrifuging the blood at 1000 to 1500 rpm for 10 minutes, the plasma and the buffy coat consisting of the lymphocytes were withdrawn and diluted with 4 parts of culture medium TC 199 (Microbiological Associates Inc.). Penicillin at 100 international units/ml and streptomycin at 100 ug/ml of culture medium were then added to the cultures. Approximately 5 drops of PHA were added for each 10 ml of culture. The cultures were incubated at 37°C.

4. The General Method of Cell Cycle Analysis:

Howard and Pelc (1953) first pointed out the existence of different periods of interphase as illustrated in Figure 1. After the completion of mitosis the cell enters a phase, called the G1 period, when no DNA synthesis occurs. Then it passes into the DNA synthetic period (S period) when nuclear DNA is replicated. This is followed by a third phase called the G2 period when again no DNA synthesis occurs. The cell is then prepared to go through mitosis. Generally, the mid point from one mitosis to the mid point of a successive mitosis is called one cell cycle.

The method for cell cycle analysis in vitro used in this study is essentially the same as used by Quastler and Sherman (1959), Wimber (1963), and Quastler (1963). In the present study only the cells in metaphase were used as the marker stage instead of all mitotic figures, as used in the studies by the workers mentioned above. The experimental procedure used in this study involved the treatment of an actively growing cell population in culture with H^3TdR for a short time (pulse labeling). Thymidine (TdR), being a DNA precursor, is incorporated into the chromosomes if the cell is in the S period or if it moves into the S period from the G1 period during the isotope administration. After the labeled thymidine is removed from the culture the cells continue to grow and pass through the rest of the cell cycle. The daughter cells may then go through a second cell cycle depending upon the developmental potential of the cells and the culture conditions. When these daughter cells replicate in the absence of labeled thymidine and move into the subsequent metaphase, the chromosomes would show radioactivity only on one chromatid (single-chromatid labeling) because of the semiconservative mode of DNA replication.

A basic concept needed for the following discussion is that cells in the later phases of the cell cycle reach mitosis before cells that are in earlier phases of the cell cycle. For example, an interphase cell labeled in the late S period would arrive at metaphase earlier than a cell labeled in the early S period. Therefore, if an actively growing population of cells is pulse labeled with H^3TdR and, thereafter, samples are harvested covering a period of time longer than one cell cycle, then the following changes in the frequency of labeled metaphase figures can be expected, as shown in Figure 2. In the initial harvests after pulse no labeled metaphase figures are expected since these metaphase cells were in the G2 period at the time of pulse labeling with H^3TdR. Then, in a later harvest labeled metaphase figures appear as the cells that were at the end of the S period during the pulse reach metaphase. In harvests subsequent to this, the frequency of metaphase labeling rises to about 100 per cent. The level of 100 per cent labeling is maintained over an extended series of harvest which is represented in Figure 2 as a plateau. In the next few harvests, the percentage of labeled metaphase figures decreases, reaching about zero per cent and being maintained at this level for several harvests. In this latter series of harvests the unlabeled metaphase figures were cells which were either in G1 period or mitosis during pulse. These cells might also have been in G2 period during pulse and then have gone through mitosis and a further cell cycle. Thereafter, the frequency of labeled metaphase cells rises again to a second plateau as the cells, which were in the S period during pulse labeling and had gone through mitosis and a subsequent cell cycle, reach metaphase. The second wave descends thereafter because of entry into metaphase of cells which were in G1 period during the pulse and then had progressed to mitosis and through the next cell cycle.

From Figure 2 the average duration of the different phases of the cell cycle can be determined. The average duration of the G2 period is defined as the time from the pulse labeling to the time when 50 per cent of the metaphase figures are labeled, that is, the median of the ascending slope of the first wave in Figure 2. The time from the median of the ascending slope of the first wave to the median of the descending slope of the first wave gives the average duration of the S period. The total cell cycle is obtained by measuring the duration from any point, for example the middle of the plateau, on the first wave of labeled metaphases to the same point on the second wave. The G1 period is estimated by subtracting the total time taken by G2 and S periods from the time taken to complete the total cell cycle.

5. Specifications of the Pulse Labeling, Serial Harvest Experiment:

In order to use the above methods for cell cycle analysis the following experiment was designed. The lymphocyte cultures were incubated for 47 to 66 hours without being disturbed for the last 12 hours so that the cells would sediment and the cell-free supernatant (preconditioned medium) could be removed and saved for later use. This supernatant was centrifuged to remove any cells that might possibly remain in it. To the medium containing the transformed lymphocytes, H^3TdR (specific activity 11.0 c/mM) at a final concentration of 3 uc/ml was added. The female cells were labeled for 15 minutes and the male cells for 8 minutes. The cells were then centrifuged, the medium containing H^3TdR removed, and the cells washed two or three times with Hanks' balanced salt solution in order to remove any H^3TdR remaining in the culture. The cells were then suspended in the preconditioned medium which was withdrawn previously. Unlabeled thymidine was added to the culture of male cells to dilute the H^3TdR pool in the cells. The culture was then subdivided into 28 samples of 5 ml each and put into smaller bottles one of which was harvested every hour or less (see Tables I and II for the exact times in the experiments using the male and female lymphocyte cultures) for the next 26 hours. At the time of harvest, the contents of each bottle were transferred to a centrifuge tube and spun for 10 minutes at 1500 rpm. The supernatant was then removed and the cells were suspended in a hypotonic solution of 0.7 per cent sodium citrate for about 10 minutes at 37°C. This causes the cells to swell, as a result of osmotic intake of water through the cell membrane, and reduces the overlapping of chromosomes in metaphase spreads on slides. After the hypotonic treatment, the cells were centrifuged again, the supernatant withdrawn, and the remaining button of cells fixed in a solution of glacial acetic acid and absolute ethanol (1:3 by volume) for 15 to 20 minutes at room temperature. At the end of this period the cells were centrifuged, the fixative removed, and the cells were treated with 45 per cent glacial acetic acid for 2-3 minutes. The cells were again centrifuged, the acetic acid withdrawn and the cells resuspended in a few drops of the fixative or in a few drops of absolute ethanol. Several drops of this cell suspension were then placed on a clean slide and ignited. This quick drying insured good spreading of the metaphase chromosomes on the slide. The cells on the slides were then stained with acetic orcein and covered by a coverslip which was then sealed with wax.

6. Obtaining Data for the Cell Cycle Analysis:

The data for the cell cycle analysis was obtained by locating and recording the co-ordinates of two-hundred metaphase cells on the slides by scanning with a microscope. Thereafter, the coverslips were removed, the acetic orcein stain was washed off with 70 per cent alcohol followed by washing with distilled water. The slides were then coated with Kodak NTB 3 liquid emulsion and exposed for 6 to 7 days before developing. Thereafter, the slides were restained with 0.3 per cent azure bromide. The metaphase figures were relocated under the microscope and scored as 'labeled' or 'unlabeled'. The frequencies of labeled metaphase figures in each harvest were plotted in a graph (Figure 3) from which the cell cycle was analyzed using the method discussed above.

7. Methods for the Estimation of the Rates of Chromosome Replication in Bovine Lymphocytes in Culture:

In this study, an attempt was made to determine the rates of replication throughout the S period of the X and Y chromosomes in the male complement and of the two X chromosomes and two longest autosomes in the bovine female complement. Essentially this was based on the assumption that the relative grain densities on chromosomes that were pulse labeled in the S period would be related directly to the relative rates of incorporation of H^3TdR (i.e., the rates of DNA synthesis) during the interval of pulse labeling (Hunte and Foote, 1967).

The experiment that was designed to estimate the rates of X and Y chromosome replication in the male was similar to the experiment which was used for the cell cycle analysis of female bovine lymphocytes. The study on the rates of replication of the two longest autosomes in the bull, however, was not attempted. The methods, for culturing, labeling with H^3TdR, harvesting, and autoradiography of the lymphocytes were the same as described earlier for the experiment with the lymphocytes from the female. Only nine serial harvests were made covering a period of 12.5 hours after pulse (see Table I for the numbers of metaphase cells obtained in these harvests).

The experiment that was designed for the cell cycle analysis was also used for the analysis of chromosome replication in the female complement. Well-spread metaphase figures with 60 chromosomes and with clearly identifiable X chromosomes were taken from the first sixteen harvests, covering the first 14 hours after pulse (Table II).

This meant that the analysis covered a duration of one full cell cycle. The selected metaphase figures were photographed and their locations on the slides recorded. The two longest autosomes in each metaphase figure were determined by measuring a number of the longest autosomes in the photograph. These two autosomes, however, were not necessarily homologous since variation in length of chromosomes did occur from one metaphase to another and since among the longest autosomes there were several of similar length. For example, the X chromosomes which could be identified unequivocally in any metaphase figure were found to be shorter than the longest autosomes in some cells and longer in other cells. Although the selected autosomes were not necessarily homologous the data did not invalidate the experiment since they were used merely as examples of euchromatic chromosomes for comparison with the X chromosomes.

The total grain density of the individual chromosomes obtained from both experiments was determined by counting the silver grains lying directly over the chromosome and in the area around the chromosome within the width of one chromatid. Due to heavy incorporation of H^3TdR, silver grains on the autoradiographs of some metaphase figures merged and formed dense blocks, complicating the individual grain counts. In such cases, the grain densities were determined by an alternate method, designed to approximate the visual counting method. In the alternate method, the average diameter of a grain (1 mm at a magnification of 2120 X) and the area of each block of grains were determined so that the number of individual grains in the block could be estimated.

The amount of background labeling which varied from slide to slide may have introduced a source of error into the grain counts. Therefore, a correction for background labeling was considered. Several metaphase figures were selected with a wide variation in background labeling of the immediately surrounding area. The number of grains in the area surrounding each of these figures were counted. The average area of an X chromosome or long autosome was also determined. From this information a range of 0.25 to 0.80 background grains was estimated to occur in the area covered by one of these chromosomes. This was a relatively low level of background labeling so no attempt was made to correct the chromosomal grain counts.

Since the X and Y chromosomes could be distinguished from each other morphologically, the method of analysis of the grain counts in the bull experiment will be discussed before the more difficult study using the cow complement where the two X chromosomes could not be distinguished from each other morphologically. The grains on the X and Y chromosomes in each harvest were counted and the average grain densities for the X and Y chromosomes calculated (Table I and Plate 6).

In Figure 4, the graphs obtained by plotting average grain densities of the X and Y chromosomes from the different harvests reflect the rates of chromosomal DNA synthesis in different parts of the S period. The cumulative amount of DNA synthesized on each chromosome as the S period progressed was obtained by the integration of these average grain density curves from the beginning of the S period to each harvest within the interval respresenting the S period. These integrals were then plotted graphically to show the relative amounts of DNA synthesized by each chromosome (Figure 5).

Unlike the sex chromosomes of the bull, the two X chromosomes of the cow could not be distinguished from each other morphologically. However, a conclusion made in studies by other investigators is that the heterochromatic X chromosome incorporates less H^3TdR than the euchromatic X chromosome and the autosomes in the early S period, but that the reverse situation exists in the late S period. Therefore, one might expect the two X chromosomes to differ significantly from each other in their grain densities in the early and late S periods. This may be tested against the hypothesis of no difference in grain densities between two X chromosomes by calculating chi-aquare values. If significant differences in grain densities occurred, then we might reasonably classify, on the basis of the grain densities, the two X chromosomes in a metaphase figure in different harvests as the euchromatic and heterochromatic X chromosomes. The pairs of X chromosomes that were shown to have a significant difference in grain densities constitute what was called the D category. However, in some cells labeled in the early and late S periods and most of the cells labeled in the mid S period, the two X chromosomes could not be distinguished individually since they had similar grain densities. Therefore, these pairs were classified as the S category. Furthermore, many metaphase figures labeled in the initial and terminal stages of the S period had two X chromosomes with a total of 9 or fewer grains which was too low a number for a chi-square test. These lightly labeled pairs constituted what was termed the L category.(see Plates 1 - 4).

In estimating the average grain densities on the euchromatic and heterochromatic X chromosomes in each harvest two methods of calculation were used. The first method gave the greatest possible difference in the average grain densities between the two X chromosomes since this method did not take into account to which of the L, S or D categories the pairs belonged. This introduced a source of error due to misclassification of the two X chromosomes into the heterochromatic and euchromatic classes because of insignificant differences in the grain densities of individual pairs. The averages obtained by this method were referred to as the uncorrected average grain densities. The second method of calculating the average grain densities did take into account to which of the L, S or D categories the pairs belonged. This method gave what were called the corrected average grain densities. The same method of analysis was also used to estimate the rates of replication of the two longest autosomes in the bovine complement. Based on the inspection of autoradiographs it was expected that with this analysis the two longest autosomes and one of the X chromosomes would have the same rates of DNA synthesis. If this expectation were realized, then the estimates of the rates of chromosomal DNA synthesis obtained by this method of analysis could be considered more reliable than if the rates of DNA synthesis on the two longest autosomes were not studied. The details of each method are described below.

In the first method, which may be easily followed in Table II, individual X chromosomes of each metaphase figure were first classified as 'heavily' or 'lightly' labeled by counting the grains on them. The actual grain counts were then entered under the 'heavily' or 'lightly' labeled X chromosome columns in Table II. The uncorrected average grain densities of the 'heavily' and 'lightly' labeled classes were then calculated and plotted (Figure 5). In doing this the following assumptions were made: (1) when a pair of X chromosomes belonged to the D category, classification as the 'heavily' and the 'lightly' labeled X chromosome sorted out the heterochromatic X chromosome from the euchromatic X chromosome, and (2) when a pair belonged to the L or S categories, the classification of individual X chromosomes of a pair as 'heavily' or 'lightly' labeled was sometimes wrong and, therefore, some error was introduced into the estimation of these uncorrected average grain densities. The same procedure was used in the case of the two longest autosomes to obtain uncorrected average grain densities (Table II and Figure 5). A comparison of the proportions of the pairs of X chromosomes that belonged to the L, S and D categories in the different harvests was made to see in which harvest the error in the uncorrected average grain densities may have been the greatest (Figures 7 and 8).

In order to adjust for the possible error mentioned above, the method for obtaining the corrected average grain densities of the two X chromosomes in each harvest was devised. The procedure also applied to the two longest autosomes. The grains counted on the individual chromosomes of the pairs in the L, S and D categories were assigned to two classes (the 'heavily' and 'lightly' labeled X chromosome classes) which corresponded with the 'heavily' and 'lightly' labeled X chromosome columns in Table II. The total grains on the heavily labeled X chromosome of each pair in the D category were assigned to the 'heavily' labeled X chromosome class. Similarly, the total number of grains on the lightly labeled X chromosome of each pair in the D category was assigned to the 'lightly' labeled X chromosome class. In the case of the S category, half of the total number of grains counted on all of the X chromosomes in a given harvest was assigned to each of the 'heavily' and 'lightly' labeled X chromosome classes. In the case of the L category, the sum of the grains on all of the X chromosomes was assigned to the 'heavily' and 'lightly' labeled X chromosome classes on the basis of the number of pairs of X chromosomes in the S and D categories in each harvest and the actual grain distribution in these categories (see following formulae). Next the total number of grains assigned to each of the 'heavily' and 'lightly' labeled X chromosome classes was divided by the total number of pairs of X chromosomes or cells in the harvest in order to obtain the corrected average grain densities of each chromosome which were plotted in Figure 9.

The entire procedure for correcting the average grain densities on the X chromosomes and on the two longest autosomes in each harvest can be summarized algebraically by the following formulae:

$$H = (A + C + D + F) \,/\, M$$
$$L = (B + C + E + F) \,/\, M$$

Where symbols mean:

H = the average number of grains on the 'heavily' labeled X chromosome or longest autosome class

L = the average number of grains on the 'lightly' labeled X chromosome or longest autosome class

A = the total number of grains on the 'heavily' labeled X chromosome or 'heavily' labeled longest autosomes in the D category

B = the total number of grains on the 'lightly' labeled X chromosomes or 'lightly' labeled longest autosomes in the D category

C = half of the total number of grains on all of the X chromosomes or on all of the two longest autosomes in the S category

If, TL = the total number of grains on all of the chromosomes of the pairs of X chromosomes or two longest autosomes in the L category

then,

$$D = TL \times \frac{A}{A+B} \times \frac{\text{number of pairs in D category}}{\text{number of pairs in D+S categories}}$$

$$E = TL \times \frac{B}{A+B} \times \frac{\text{number of pairs in D category}}{\text{number of pairs in D+S categories}}$$

$$F = TL \times \frac{1}{2} \times \frac{\text{number of cells in S category}}{\text{number of cells in D+S categories}}$$

Also,

M = the total number of metaphase cells in the harvest

It is possible that the correction procedure over-compensates for the error introduced by classifying chromosomes of a pair as 'heavily' and 'lightly' labeled, especially in the case of the X chromosomes. For instance, although the grain density difference within a pair may be insignificant statistically, this difference may yet be a real reflection of differential rates of the H^3TdR incorporation between the heterochromatic and euchromatic X chromosomes during the pulse. Therefore, one may think of the corrected average grain densities as an estimate of the least difference in the rates of replication between the chromosomes of a pair. Most likely, the relative rates of replication are between the estimates obtained from the corrected and uncorrected average grain densities.

A further step in the analysis was suggested by the graphs of the uncorrected and corrected average grain densities of the two longest autosomes and the two X chromosomes (Figures 6 and 9). In this graph it could be seen that in the early S period the 'lightly' labeled X chromosome was labeled much less than the other X chromosome and the two autosomes. These last three chromosomes were labeled about the same. In the mid S period, all four chromosomes were labeled with about the same intensity. In the late S period, one X chromosome was found to be more heavily labeled than the other X chromosome and the two longest autosomes. It was then thought that the 'lightly' labeled X chromosome in the early S period was the same X chromosome that was 'heavily' labeled in the late S period. In other words, the graph of this X chromosome was represented by the average grain densities of the 'lightly' labeled X chromosome in the first half of the S period and by the average grain densities of the 'heavily' labeled X chromosome in the second half of the S period. Furthermore, the graph of the other X chromosome was represented by the average grain densities of the 'heavily' labeled X chromosome in the early S period and by the average grain densities of the 'lightly' labeled X chromosome in the late S period. This 'switch-over' in the sequence of average grain densities was supported by the integration of the curves from the beginning to the end of the S period before and after the 'switch-over'. It was expected that some of these integrals should be equal because theoretically the two X chromosomes synthesize an equal amount of DNA in a complete S period. This hypothesis of no difference between integrals was tested by calculating chi-square values. It was found that equal integrals were obtained only if the 'switch-over' was made. In the case of the two longest autosomes there was no obvious 'switch-over' point, so this procedure was not applied.

The cumulative amounts of DNA synthesized on the chromosomes as the S period progressed was estimated by integrating the uncorrected and corrected average grain density graphs (Figures 6 and 9) from the beginning of the S period to subsequent points in the S period. The series of integrals obtained were then plotted to give uncorrected and corrected graphs of the total amount of DNA synthesized by the two X chromosomes (Figure 10) and the two longest autosomes (Figure 11). The cumulative integrals up to the end of the S period for the two longest autosomes and the two X chromosomes were all expected to be equal since these chromosomes are the same size and should have synthesized equal amounts of DNA in the S period. Therefore, each possible combination of pairs of cumulative integrals for the entire S period were tested for equality by a chi-square test.

In order to determine which of the chromosome graphs in Figures 10 and 11 were similar and which were different, a correlation analysis and linear regression analysis were made. They were based on a comparison of the first derivatives of any two graphs at similar harvest times. Since the average grain densities were also a measure of the first derivatives, these values were used in the analyses which were carried out using a suitable computer program. In the correlation analysis if no correlation was found between the average grain densities of any two chromosomes, this would imply that the rates of DNA synthesis on the two chromosomes in question did not vary together and that their cumulative integral graphs in Figures 10 and 11 would also be significantly different. If a significant correlation was found, this would imply that the rates of replication did vary together in some way. The linear regression analysis was then made to establish whether the correlation reflected a 1:1 relationship in the average grain densities of each combination of two chromosomes. The linear regression analysis was first made using the uncorrected average grain densities and then using the corrected average grain densities.

If a 1:1 relationship were found to exist, this would imply that the rates of DNA synthesis of the two chromosomes were the same in various parts of the S period and that the cumulative integral graphs (Figures 10 and 11) were essentially the same.

The cumulative integral graphs (Figures 10 and 11) showing the relative amounts of DNA (expressed in grain-hours) synthesized by each chromosome as the S period progressed were converted to the graphs in Figure 12 showing the percentage of DNA synthesis completed by each of the X chromosomes and each of the two longest autosomes as the S period progressed. This conversion enables one to make statements about the proportions of DNA synthesized in various intervals of the S period by each chromosome.

8. Methods of Studying the Patterns of Initiation of DNA Synthesis in Chromosomes of Bovine Lymphocytes:

The metaphase figures from the experiments that were designed to estimate the rates of replication of each of the two X chromosomes and the two longest autosomes from the bovine female and of the X and Y chromosomes from the bovine male were also used in the initiation studies. Since no colchicine was added to the cultures and the duration of mitotic metaphase is very brief, the majority of metaphase figures collected from each harvest must represent cells that were at the same phase of the cell cycle at the time of pulsing. Furthermore, according to the cell cycle analysis made during the present investigation, an average of 10 hours is required for bovine lymphocytes, labeled at the very beginning of the S period to come to metaphase. Therefore, by determining the labeling patterns of the chromosomes in the majority of metaphase figures obtained from harvests made between 11 and 8 hours after pulse treatment, one should be able to study the DNA synthetic activities of the sex chromosomes and the autosomes, at the initial stages of the S period.

IV. RESULTS

1. Results of the Cell Cycle Analysis:

Two-hundred metaphase figures from each harvest after pulse labeling were examined in order to determine the percentage of labeled metaphase figures in each harvest. It can be seen in Table III and Figure 3 that in the harvest at 1 hour and 40 minutes after pulse, 25 per cent of the metaphase figures were found to be labeled. These labeled metaphase cells were at the very end of the S period during the isotope treatment. This observation implies that one quarter of the lymphocytes in metaphase in this harvest had a G2 period of 1 hour and 40 minutes or less. This, therefore, was the minimum estimate of the G2 period for this population. The frequency of labeled metaphase figures increased rapidly thereafter and in the culture harvested 2 hours after pulse, 40 per cent of the metaphase figures were found to be labeled. This gave the average duration of the G2 period. The frequency of labeled metaphase figures ascended to about 100 per cent at 4 hours after pulse. This range from 1 hour and 40 minutes to 4 hours indicated the variation in the duration of G2 period that existed among the lymphocytes. The extent of metaphase labeling remained at about the 100 per cent level from 4 to 8 hours after pulse, suggesting that only the cells that were in the S period during the isotope treatment had entered metaphase during this period of harvesting. Four hours, therefore, was the minimum estimate for the duration of the S period. In subsequent harvests made between

8 and 11.5 hours after pulse, the frequency of labeled metaphase figures decreased gradually and in the harvest made 10 hours after pulse, 50 per cent of the figures were found labeled. This descending curve was caused by the entry of cells, that were in G1 period during the isotope treatment, into the subsequent metaphase. The frequency of labeled figures reached a minimum point of 44 per cent at 11.5 hours after pulse. The time interval between the median of the ascending slope and the median of the descending slope of the first wave gave an estimate of 8 hours for the duration of the average S period in the lymphocyte population used in this experiment.

Subsequent to the harvest at 11.5 hours, the frequency of labeled figures gradually increased again and reached a plateau at 65 per cent between 16 and 20 hours after pulse. In the harvest made at 26 hours after labeling the frequency of labeled figures dropped to about 38 per cent. The second wave of labeled figures occurred by the entry of the labeled cells into the second metaphase after pulse. The duration of the total cell cycle, which is about 12 hours, was estimated by determining the time interval between the midpoint of the plateau of the first wave (at 6 hours after pulse) and the same point of the second wave (at 18 hours after pulse). By subtracting the total time taken by the majority of cells to complete the G2 and S periods (2 + 8 hours), from the average time taken by lymphocytes to complete one cell cycle (12 hours), the duration of the G1 period was determined. This gave an estimated average G1 period of 2 hours.

It should be pointed out that, since no colchicine was added to the cultures and the duration of metaphase is very short, the metaphase figures collected from each harvest must have represented a population of cells that were more or less synchronous in the sense that they were labeled in the same parts of the S period. The majority of labeled metaphase figures obtained from earlier harvests must have been cells which were in contact with the isotope at the end of the S period and the labeled figures from later harvests must have been cells that were in earlier parts of the S period.

As can be seen in Figure 3, the second wave of labeled metaphase figures was different from the first wave. At about 11.5 hours after pulse the frequency of labeled metaphase cells was at its minimum level of 44 per cent which was much higher than the expected level of zero per cent. Also, the second wave reached a plateau at the 65 per cent level between 16 and 20 hours after pulse and not at the 100 per cent level expected. The slopes of the second wave on either side of the plateau were not as steep as the slopes of the first wave. The second wave had a duration of at least 14 hours compared with 11.5 to 12 hours for the first wave. Furthermore, the graph of the actual frequencies of labeled metatphase figures (Figure 3) is different in certain aspects from the graph expected (Figure 2).

These differences between the first and second waves of labeled metaphases could be explained by the existence of variation among cells in the duration of the cell cycle that was shown by the cell cycle analysis. But before explaining how this variation affected the shapes of the two waves one further source of evidence for this variation is given.

It was observed that out of 200 metaphase figures from the harvest at 14 hours after pulse, which contributed to the beginning of the second wave, 6 showed an X chromosome that was conspicuously more heavily labeled than the rest of the complement, 5 showed an X chromosome that was labeled less than the rest of the complement (Plate 5), and the other figures were uniformally labeled (Table III). It could be seen in Table III that 19 per cent of the metaphase figures obtained from the harvest at 2 hours after pulse showed a conspicuously heavily labeled X chromosome (Plate 1). Such figures must have represented cells that were in the late S period during the pulse, since they were among the first cells to appear labeled in metaphase. Another noticeable characteristic of the first wave of metaphase figures was the occurence of metaphase figures with a relatively lightly labeled X chromosome in the harvests made 8 and 9 hours after pulse (Plate 2). One may suggest that the few cells at 14 hours after pulse with the pattern of differential labeling of the X chromosome seen in the late S period had passed through to metaphase, completed the subsequent cell cycle and arrived at the second metaphase after pulse by 14 hours. The few cells at 14 hours after pulse with the differential labeling pattern of the X chromosomes seen in the early S period were likely cells that were relatively slow in completing the remainder of the S and G2 periods before having been harvested in the first metaphase after pulse 14 hours later. On the average, cells of this second type complete these phases in 10 hours. The rest of the metaphases with uniform labeling represent cells that were labeled in the many parts of the S period where there was uniform incorporation of H^3TdR (Table III and Plate 3). Thus, the duration of the periods of the cell cycle may vary from cell to cell.

The observations mentioned above establish reasonably well the existence of variation in the duration of the cell cycle among individual cells of a population. It is then possible to understand that when metaphase cells are harvested at increasingly later times after pulse they represent cells that were in an increasingly broader spectrum of the cell cycle. For example, in the harvest made 2 hours after pulse, a mixture of unlabeled and labeled metaphase figures was obtained which, during the pulse, had been in G2 and S period, respectively. On the other hand, in the harvest at 18 hours after pulse labeling, the metaphase population consisted of cells that were in many parts of the G1, S and G2 periods during pulse labeling. The mixture of labeled and unlabeled metaphases had become so great that 100 or zero per cent labeling of metaphases could not be obtained in harvests in the second wave but instead only frequencies that varied between 45 and 65 per cent were observed. Furthermore, the broader second wave, as compared with the first wave, suggested that there were more cells that were slower in completing the stages of the cell cycle than there were cells which were faster than the majority of the population.

A further difference was observed between the metaphase figures of the first and second waves. Figures in the first wave were found to be labeled on both chromatids while figures in harvests belonging to the second wave were sometimes seen to have grains on only one chromatid (Plate 5). When a cell incorporates labeled DNA precursors (for example H^3TdR) into its chromosomes and completes its first division, whole-chromosome labeling is observed. The daughter cells then undergo another cell cycle in the absence of labeled precursors, so that only one of the two chromatids will be labeled in the subsequent metaphase. For this reason it was expected that most of the metaphase cells forming the second wave would have shown single-chromatid labeling. However, the frequency of such cells never went beyond 13 per cent (Table III). One possible reason for this discrepancy could be that the labeling intensity of many metaphase figures was not always high enough to distinguish the cells with single-chromatid labeling from the cells with whole-chromosome labeling. Another explanation for this discrepancy could be that some of the metaphase figures in the second wave had only reached the first metaphase after pulse since they were relatively slow in completing the stages of the cell cycle.

Metaphase figures with single-chromatid labeling were first observed in the harvest at 15 hours after pulse (Table III). The cells to first show this pattern were in the late S period at the time of pulse labeling and entered the second metaphase after pulse labeling. In other words, they grew through successive G2 + G1 + S + G2 periods, which amounts to 2 + 2 + 8 + 2 = 14 hours, using the estimates of these periods obtained previously from the cell cycle analysis. The significance of the first appearance of single-chromatid labeling at 15 hours after pulse is that the time of observation corresponds well with the prediction of 14 hours after pulse made above. Therefore, the estimates of the periods of the cell cycle could be considered fairly reliable.

2. Results of the Analysis of Rates of Replication of the X and Y Chromosomes in Cultured Lymphocytes from Bovine Male:

In order to determine the DNA synthetic activities of the X and Y chromosomes at different phases of the S period, the silver grains overlying these chromosomes in metaphase figures obtained from various harvests were counted on the autoradiographs and the average grain densities of the X and Y chromosomes in each harvest were calculated. These results are presented in Table I. The average grain densities for the X and Y chromosomes from each harvest were then plotted to obtain Figure 4. Assuming that the average duration of the different phases of the cell cycle of the lymphocytes from the bovine male was comparable with the duration of these periods in the female lymphocytes, then the majority of the metaphase figures obtained from the harvest made 10 hours after the pulse represent cells that were at the beginning of the S period at the time of the isotope treatment. The figures from the harvest at 2 hours were at the end of the S period. Harvests in between these represent the intermediate parts of the S period. Unfortunately, harvests were not made within the first 3.5 hours after pulse. As seen in Figure 4, the average number of grains on the X chromosomes in metaphase figures obtained from the harvest at 10 hours was approximately 1.9 grains; from the harvest at 6.5 hours, 4.8 grains; and from the harvest at 3.5 hours, 1.5 grains. The average grain densities on the Y chromosome in the same harvests after pulse were approximately 0.5, 2.3 and 1.4 grains, respectively.

The graphs of the average grain densities on the X and Y chromosomes in Figure 4 were then integrated from the beginning of the S period to each of the points in the S period represented by a harvest. This series of integrals, when plotted in Figure 5, gave a profile of the cumulative and relative amounts of DNA synthesized by each chromosome as the S period progressed. The graphs in Figure 5 suggested that the X chromosome of the male replicated linearly throughout the S period. The Y chromosome began replication in the first half of the S period at very low rates but accelerated in its rate of DNA synthesis in the second half of the S period, when most of its DNA was synthesized.

3. Results of the Analysis of the Rates of Replication of Chromosomes in the Bovine Female Complement:

The data for the study of the rates of replication of the two X chromosomes and the two longest autosomes in the bovine female complement were obtained from the first 17 of the total 28 serial harvests used for the cell cycle analysis. The grain counts on the heavily labeled X chromosome and the lightly labeled X chromosome in each metaphase figure were listed in separate columns in Table II. In a similar manner, the grain counts on each of the two longest autosomes in each metaphase figure were listed in two columns (Table II). It was hypothesized that there was no significant difference in the grain densities between the members of each chromosome pair. On the basis of a chi-square test of this hypothesis, each pair was classified as either L, S or D category, as explained in Methods. The uncorrected average grain densities were then calculated for each column in Table II. These averages (uncorrected averages) were then plotted graphically in Figure 6. The part of this graph dealt with in this analysis was the interval from 10 to 2 hours after pulse representing the S period as determined by the cell cycle analysis. Certain differences between chromosome classes were noted upon inspection of this graph. It could be seen that the average grain density on the 'heavily' labeled X chromosomes in the late S period (3 to 2 hours after pulse) was considerably higher than that on the 'lightly' labeled X chromosome and that on the longest autosomes. For example, at 3 hours after pulse the 'heavily' labeled

X chromosomes had an average of 44 grains compared with 19-26 grains on the 'lightly' labeled X chromosomes and the longest autosomes. In the early S period, (10 to 7 hours after pulse) the 'lightly' labeled class of X chromosomes was noticeably more lightly labeled than the other three classes of chromosomes. This 'lightly' labeled class showed an average grain density of 3 grains at 9 hours after pulse and of 10 grains at 8 and 7 hours after pulse as compared with the other three chromosome classes with average grain densities ranging from 8-13 grains at 9 hours to 19-25 grains at 8 hours after pulse. In the mid S period from 6 to 4 hours after pulse, all four chromosome classes showed similar average grain densities, ranging from 16 to 28 grains.

As mentioned in Methods, the uncorrected average grain densities most likely contained some error due to the classification of the individual chromosomes, belonging to the pairs in the L and S categories, as 'heavily' or 'lightly' labeled. The extent of this error was expected to be greatest when a large proportion of the chromosome pairs in any given harvest were classified in the L and S categories rather than in the D category. Therefore, in order to determine in which harvests the error was greatest, the proportions of the L, S and D categories in each harvest were calculated and plotted in Figure 7 for the X chromosomes and in Figure 8 for the autosomes.

In the case of the L category, the graph for the X chromosomes (Figure 7) and the graph for the longest pair of autosomes (Figure 8) were similar. At the beginning of the S period, represented by the harvest at 10 hours after pulse, 30 to 40 per cent of the chromosome pairs under question were classified in the L category. This frequency gradually decreased to zero per cent in the mid S period (6 hours after pulse) and subsequently rose to 25 to 35 per cent at the end of the S period (2 hours after pulse).

Thirty-five per cent of the metaphase figures labeled in the early S period (10 to 8 hours after pulse) had pairs of X chromosomes belonging to the S category (Figure 7). In the mid S period (7 to 4 hours after pulse) the frequency increased to more than 55 per cent and reached a maximum of 100 per cent at 6 hours. In the late S period (3 to 2 hours after pulse) the frequency was less than 10 per cent. In the case of the two longest autosomes (Figure 8), the majority of the pairs were classified in the S category throughout the entire S period; the frequency ranged between 55 and 90 per cent.

The frequency of the longest autosomal pairs classified in the D category ranged from 5 to 25 per cent throughout the S period (Figure 8). The frequency of X chromosome pairs in the D category (Figure 7) varied throughout the S period. In the early S period (10 to 7 hours after pulse) pairs of X chromosomes in the D category constituted between 15 and 40 per cent of the population. In this interval, one of the X chromosomes was labeled much less than both the other X chromosome and the autosomes, and sometimes it was practically unlabeled. At 6 hours after pulse, in the mid S period the pairs of X chromosomes belonging to the D category reached zero per cent. In the late S period (5 to 2 hours after pulse), there was a gradual increase in the frequency of the D category, reaching about 70 per cent at 2 hours. In this interval, one X chromosome was more heavily labeled than the other chromosomes of the complement.

In summary, the X chromosome pairs belonging to the S category were found to be the most prominent category in the mid S period. Therefore, a correction of the average grain densities of the two X chromosome classes appeared to be especially necessary in the mid S period. A correction was also needed for the calculation of reliable average grain densities of each of the autosomal classes throughout the entire S period since most pairs belonged to the S category in all parts of the S period. Furthermore, since the L category constituted about a third of the pairs of X chromosomes and of the pairs of longest autosomes in harvests at the beginning and at the end of the S period, the correction was needed to obtain more accurate average grain densities of the chromosomes in these harvests.

The corrected average grain densities on each of the X chromosomes and each of the two longest autosomes were plotted graphically in Figure 9. The corrected average grain densities of the two longest autosomes did not differ from one another by more than 2 or 3 grains at any point in the entire S period. The corrected averages of the two longest autosomes were about 11 grains at the beginning of the S period (10 and 9 hours after pulse) and at the end of the S period (2 hours after pulse). In the mid S period (8 to 3 hours after pulse), however, the corrected averages varied between 16 and 25 grains. In the early S period (10 to 7 hours after pulse), one X chromosome was labeled with nearly the same intensity as the two longest autosomes (only 0-4 grains less than the autosomes). The other X chromosome was labeled much lighter than the other three chromosomes (4-10 grains less). In the mid S period (6 to 4 hours after pulse), each of the four chromosomes had about the same average grain density ranging from 18-26 grains. In the late S period (3 to 2 hours after pulse), one X chromosome was more heavily labeled than the other three chromosomes. The 'heavily' labeled X chromosome had 42 grains at 3 hours and 29 grains at 2 hours after pulse, whereas the other three chromosomes showed 21-25 grains at 3 hours and 8-13 grains at 2 hours after pulse.

On examining the graphs of the uncorrected and corrected average grain densities in Figures 6 and 9, it appeared likely that the X chromosome which had relatively <u>low</u> average grain densities in the first half of the S period (10 to 6 hours after pulse) might be the same X chromosome which had relatively <u>high</u> grain densities in the second half of the S period (6 to 2 hours after pulse). This X chromosome was called the 'late' replicating X chromosome. On the other hand, it seemed likely that the X chromosome which had relatively high average grain densities in the first half of the S period might be the same X chromosome which had relatively low grain densities in the second half of the S period. This X chromosome was called the 'early' replicating X chromosome. On the basis of these observations, the 6-hour point in the graphs was called the 'switch-over' point.

Support for the 'switch-over' concept was obtained from the examination of the cumulative integrals of the average grain density graphs from the beginning to the end of the S period. The cumulative integrals gave an estimate of the total amount of DNA synthesized in the S period by each chromosome. Furthermore, since the four chromosomes used in the analysis were the same size, it was expected that they should synthesize equal amounts of DNA in the total S period and should have equal cumulative integrals. The cumulative integrals were obtained from the graphs of the uncorrected and corrected average grain densities on each of the X chromosomes (Figure 6) both before and after the 'switch-over'. The hypothesis that there was no significant difference between cumulative integrals was tested by calculating chi-square values. Before the 'switch-over' in the graphs of the uncorrected average grain densities of the X chromosomes, the cumulative integrals of the heavily labeled X chromosome and the lightly labeled X chromosome were found to have a ratio of 65:35. Similarly, using the integrals of the corrected average grain density graphs (Figure 9), the ratio of the heavily labeled X chromosome integral to the lightly labeled X chromosome integral was found to be

60:40. Both of these ratios were found to be significantly different from a 50:50 ratio. Therefore, graphs of the X chromosomes before the 'switch-over' were not considered to represent accurately the replicative behavior of the X chromosomes. After the 'swtich-over' the X chromosomes were referred to as the 'late' and 'early' replicating X chromosomes. The ratio of the cumulative integral of the 'late' replicating X chromosome to the cumulative integral of the 'early' replicating X chromosome was found to be 54:46 in both the uncorrected and corrected average grain density graphs. This ratio was not significantly different from the expected 50:50 ratio. Therefore, the graphs of the X chromosomes after the 'switch-over' procedure gave a more accurate representation of the replicative behavior of the X chromosomes. Correction of the average grain densities was not necessary to obtain equality of the cumulative integrals covering the entire S period in the case of the X chromosomes.

The cumulative integrals of the two longest autosomes from the beginning to the end of the S period were found without the 'switch-over', since there was no obvious point of 'switch-over' (Figures 6 and 9). The cumulative integrals of the graphs of uncorrected average grain densities of the heavily and lightly labeled autosomes (Figures 6) were found to have a ratio of 59:41 which was significantly different from a 50:50 ratio. The integrals of the corrected average grain density graphs of the heavily and lightly labeled autosomes (Figure 9) were found to have a ratio of

53:47 which was not significantly different from a 50:50 ratio. Therefore, in the case of the autosomes, the correction formulae were necessary in order to obtain equivalent cumulative integrals over the entire S period.

With the above information it was possible to attempt to estimate how much DNA was synthesized by each chromosome as the S period progressed. This was done by obtaining a series of cumulative integrals of the uncorrected and corrected average grain density graphs of each chromosome from the beginning of the S period to each point in the S period. The cumulative integrals were expressed in units of grain-hours. These cumulative integrals were plotted to show the amount of DNA synthesized by each of the X chromosomes (Figure 10) and by each of the two longest autosomes (Figure 11) as the S period progressed. In Figure 10, the graphs of the 'early' replicating X chromosome were linear suggesting that the same amount of DNA was synthesized by this chromosome in equivalent intervals in the S period. The graph of the 'late' replicating X chromosome shows that it synthesized little of its DNA in the early S period but gradually accelerated its rate of replication so that most DNA replication occurred in the second half of the S period. The final cumulative integrals of each X chromosome at the end of the S period were expected to be equal since each X chromosome synthesizes the same amount of DNA in a complete S period. The final cumulative integral of 155 grain-hours obtained for the 'late' replicating

X chromosome and the final cumulative integral of 133 grain-hours for the 'early' replicating X chromosome were found to be statistically similar. The fact that the cumulative graphs obtained from integration of the uncorrected and corrected average grain density graphs coincided very well, suggested that the correction procedure was not essential for understanding the rates of replication and the amount of DNA synthesized as the S period progressed in the X chromosomes.

In Figure 11 the cumulative graphs, obtained by integrating the uncorrected and corrected average grain density graphs of each of the two longest autosomes, were found to be linear suggesting that the rate of DNA synthesis on these autosomes was constant throughout the S period. The two cumulative integral graphs obtained from the corrected average grain density graphs probably represented the replicative behavior of these autosomes more accurately than the two cumulative integral graphs obtained from the uncorrected average grain density graphs. This was thought to be so because the final cumulative integrals for the two longest autosomes at the end of the S period using the corrected average grain densities were found to be 159 and 143 grain-hours, values which were not significantly different. On the other hand, using the uncorrected average grain densities, the final integrals of 178 and 126 grain-hours were found to be significantly different.

The cumulative integral graphs of the 'early' replicating X chromosome (Figure 10) and the two longest autosomes (Figure 11) were linear and appeared to coincide. Since these three chromosomes are euchromatic, it was suggested that a property of euchromatin is that it has a constant rate of DNA synthesis throughout most of the S period. Furthermore, the graphs of the 'late' replicating X chromosome did not appear to coincide with the graphs of the three euchromatic chromosomes described above. Since this 'late' replicating X chromosome has been shown by others to be composed of heterochromatin, it was suggested that a property of heterochromatin is that it gradually accelerates from a relatively low rate of replication in the early S period to a relatively high rate in the late S period.

The above suggestion concerning the rates of replication of euchromatin and heterochromatin was supported by correlation and linear regression analyses based on a comparison of the first derivatives of the different pairs of cumulative graphs at similar times. Since the average grain densities were also a measure of the first derivatives, these values were used in the regression analysis which was carried out using a suitable computer program.

No significant correlation was found between the uncorrected average grain densities of the 'late' replicating X chromosome and the uncorrected average grain densities of each of the other three chromosomes. Similarly, no correlation was found in the same comparisons using the corrected average grain densities. This result implied that the average grain density graphs and the cumulative integral graphs of the 'late' replicating X chromosome (Figures 6, 9 and 10) were significantly different from the graphs of the other three chromosomes (Figures 6, 9, 10 and 11).

On the other hand, a significant correlation was obtained when the uncorrected average grain densities of the 'early' replicating X chromosome and those of each of the two longest autosomes were compared in the three possible pair-wise combinations. Similarly, a significant correlation was found when the corrected average grain densities were used. With these correlations a linear regression analysis was made, based on the hypothesis that the slope (b) of the regression line would not be significantly different from 1. This would mean that the average grain densities varied together with time in a 1 to 1 relationship. The results of this regression analysis were summarized below with the slopes (b) of the regression lines obtained from each of the different comparisons of the average grain densities.

Uncorrected average grain density comparisons		Slope (b) of the regression line with standard errors
'Early' replicating X chromosome	vs. autosome A	0.847 ± 0.166
'Early' replicating X chromosome	vs. autosome B	0.766 ± 0.165
Autosome A	vs. autosome B	0.910 ± 0.061

Corrected average grain density comparisons		Slope (b) of the regression line with standard errors
'Early' replicating X chromosome	vs. autosome A	0.830 ± 0.138
'Early' replicating X chromosome	vs. autosome B	0.782 ± 0.139
Autosome A	vs. autosome B	0.938 ± 0.064

None of the above slopes was found to be significantly different from a value of 1. This meant that the data was explained well by the hypothesis that the rate of replication of the 'early' replicating X chromosome was the same as the rate of replication by each of the two longest autosomes. It also meant that the various graphs of the 'early' replicating X chromosome and of the two longest autosomes were essentially the same (Figures 6, 9, 10 and 11).

The aim of the next step in this analysis was to determine the percentage of replication completed by the different chromosomes in various parts of the S period. Using the cumulative graphs (Figures 10 and 11), the amount of DNA synthesized up to a particular point in the S period was expressed as a percentage of the total amount of DNA synthesized by each chromosome in the S period. In Figure 12, it was observed that each of the euchromatic chromosomes synthesized equivalent proportions of their DNA in equivalent intervals of the S period. On the other hand, the heterochromatic 'late' replicating X chromosome replicated a quarter of its DNA in the first two quarters of the S period, a quarter of its DNA in the third quarter of the S period, and one half of its DNA in the final quarter of the S period.

4. Results of the Study on the Initiation of DNA Replication in Bovine Chromosomes:

In the uncorrected and corrected average grain density graphs (Figures 6 and 9) it could be seen that in the case of the bovine female, both X chromosomes in metaphase figures were labeled in harvests made at 11, 10, 9 and 8 hours after pulse which covered the initial stages of the S period. This suggests that the two X chromosomes and the two longest autosomes initiate their DNA synthesis at the beginning of the S period. However, while the uncorrected average grain densities on each of the two longest autosomes and on one of the X chromosomes ranged between 5 and 9.5 grains in metaphase figures obtained from the harvest at 11 hours after pulse, between 9 and 15 grains at 10 hours, between 7.5 and 13 grains at 9 hours and between 19 and 25 grains at 8 hours; the uncorrected average grain densities on the other X chromosome at the same times were much lower at 2, 4, 3 and 9.5 grains, respectively. The corrected average grain densities on one of the X chromosomes and each of the two longest autosomes ranged between 5.8 and 8 grains at 11 hours, between 8 and 13.5 grains at 10 hours, between 7 and 11 grains at 9 hours and between 18 and 23 grains at 8 hours. The corrected average grain densities on the other X chromosomes for the same harvests were 3, 5, 4.5 and 11 grains, respectively. In a small number of metaphase figures from these harvests one X chromosome was found to be the only unlabeled chromosome in the whole complement (Plate 2). The exact number of such metaphase figures out of 200 that were examined from each of the harvests made at 11, 10, 9 and 8 hours after pulse were 1, 0, 5 and 15 figures, respectively (Table III).

The serial harvests from the lymphocyte culture of the bovine male pulse labeled with H^3TdR did not yield a large number of well-spread metaphase figures that could be used for analysing the patterns of initiation of DNA replication in the bovine male complement. Furthermore, the grain densities on the autoradiographs of most of these metaphase figures were very low (Plate 4 and Table I). However, even such low grain densities were considered to reflect a real incorporation of H^3TdR because there was essentially no background labeling in these autoradiographs. Therefore, the average grain densities of the X and Y chromosomes in metaphase figures obtained from harvests between 11 and 8 hours after pulse were examined to determine the patterns of initiation of DNA replication in the X and Y chromosomes (Figure 4). The X chromosome had an average of 0.7, 0.8, 1.8, 1.6 and 3.3 grains in harvests made at 11, 10.5, 9.5 9 and 8 hours after pulse, respectively. The Y chromosome was found to have an average of 0.2, 0.3, 0.5, 0.1 and 0.5 grains for the same harvests, respectively. These observations suggest that the X chromosomes and the two longest autosomes in the bovine female and the X and Y chromosomes in the male initiate replication simultaneously at the beginning of the S period.

V. DISCUSSION

The average duration of the G1, S and G2 periods of cow lymphocytes in culture was found to be 2, 8 and 2 hours, respectively, and the duration of the total cell cycle was found to be about 12 hours (Figure 3). The estimation of the average duration of the various phases of the cell cycle is essential for studying the replicative behavior of the individual chromosomes throughout the S period of any given complement. Since cells can incorporate H^3TdR only during the S period and the individual chromosomes can be studied only at metaphase, the amount of time needed for cells labeled at various stages of the S period to reach metaphase must be estimated. In this study the average duration of the S and G2 periods were estimated to be 8 and 2 hours, respectively. Therefore, the majority of metaphase cells from harvests made at 10.5, 10 and 9 hours after pulse were in the initial stages of the S period. The labeled metaphases in harvests from 8 to 4 hours after pulse were estimated to be in the mid S period during the pulse. The harvests at 3 and 2 hours after pulse had metaphase cells that were labeled in the terminal stages of the S period. Furthermore, since some variation does occur in the duration of the periods of the cell cycle among cells from various tissues or among cells of the same tissue, the average duration of different phases of the cell cycle must be analyzed for the cell culture being used in order to study patterns and rates of replication of individual chromosomes. For example, an epithelial cell line established from calf liver was found to have a slightly longer cell cycle consisting of G1, S and G2 periods of 16, 8 and 6 hours duration, respectively (Kuyper _et al_., 1962). Gartler and Burt (1964) showed that in cells from short term fibroblast cultures derived from calf kidneys, the average duration of the S period was slightly more than 8 hours and of the G2 period, 3.5 hours. Both of these estimates differed somewhat from the estimates made for the cow lymphocyte culture used in this study.

The results of the present study are also of value for a comparative study of the duration of the various phases of the cell cycle in cells from such mammalian species as: Chinese hamster, mouse, rat, mastomys and dog (see Appendix). The duration of the S period in cells of these species is about 8 hours, plus or minus 1 or 2 hours. The duration of the G1 period ranges from 1 to 6 hours or more and the length of the G2 period varies from 1 to 8 hours but the average is about 3 hours. The duration of the total cell cycle ranges between 9 and 24 hours, but averages about 12 hours. These cell cycles are quite similar to the cell cycle of the cow lymphocytes. It seems likely that these approximately 12 hour cell cycles may have been selected in the process of evolution to enable the organism to adapt to the daily cycle.

The cell cycle analyses of various human cell populations showed that the duration of the G1, S and G2 periods are approximately 6-8, 8 and 3-6 hours respectively (see Appendix). The total cell cycle requires about 20 hours. These estimates deviate from the estimates of the duration of the cell cycle in the species mentioned above.

The variations in the duration of the different periods of the cell cycle in cells from different mammalian species or among cells from the same species may be due to environmental factors such as the different cultural conditions and temperature fluctuations and to genetic factors and the aging of the cells.

Another finding of these experiments is that both X chromosomes and the two longest autosomes in the bovine female complement initiate DNA synthesis at the beginning of the S period, since all four chromosomes were found to be labeled in metaphase figures obtained from the harvests made between 11 and 8 hours after pulse. However, at the beginning of the S period one X chromosome was found to replicate at a considerably lower rate than the other X chromosome and the two longest autosomes. In a few of the metaphase figures labeled in this initiation interval, some metaphase figures were found in which one X chromosome was the only unlabeled or very lightly labeled chromosome in the complement (Plate 2). This suggested that both X chromosomes in the bovine female complement began replication simultaneously but after continuous synthesis of DNA by both for a period of time, either the rate of DNA synthesis in one X chromosome dropped significantly or DNA synthesis underwent a total cessation for a short period of time (Mukherjee _et al_., 1968). This period of reduced DNA synthesis may have occurred about 2 hours after the beginning of the S period as suggested by the results shown in Table III, where the largest sample of such metaphase figures (15 out of 200 metaphase figures) was found at 8 hours after pulse. However, this interruption of DNA synthesis may have occurred only in a limited number of cells as suggested by the low frequency of cells with the labeling pattern mentioned above, or the duration of interruption was shorter than the interval of pulse labeling with H^3TdR. Furthermore, the average grain densities (Figures 6 and 9) of one of the X chromosomes did not drop between 11 and 8 hours as expected by this hypothesis of reduction or cessation of DNA synthesis. Perhaps these cells with an unlabeled X chromosome were simply extremely variable cases of the differential labeling between the two X chromosomes in the early S period.

The two X chromosomes were reported to initiate replication simultaneously at the beginning of the S period in previous studies which involved a continuous labeling technique and cultured lymphocytes from normal females (Mukherjee and Sinha, 1965), from a human female with an X/iso-X sex chromosome constitution (Mukherjee _et al_., 1966), and from bovine females (Mukherjee _et al_., 1967). Similar observations were also made by a number of other investigators using various mammalian species, (Evans _et al_., 1965, in the mouse; Bianchi, 1966b, in the rat; Brown _et al_., 1966, in the dog; Gartler and Burt, 1964, in cattle; and Bianchi and De Bianchi, 1965, in man). On the other hand, a number of studies suggest that the X chromosome which synthesizes its DNA at a higher rate at the end of the S period is late in initiating DNA synthesis (Graves, 1967, in the kangaroo and wallaby; Schneider and Reike, 1967, in the opossum; Taylor, 1960, and Hsu, 1964, in the Chinese hamster; Chang *et al*., 1965, and Bianchi and De Bianchi, 1966a, in the rat; Huang, 1968, in *Rattus* (*mastomys*) *natalensis*; and in man, Morishima *et al*., 1962, Gilbert *et al*., 1962, Petersen, 1964, German, 1964, Hsu and Lockhart, 1964, Cave, 1966, Comings, 1967b, Ockey *et al*., 1966, Priest *et al*., 1967, Sofuni and Sandberg, 1967, and Takagi and Sandberg, 1968a,b)..

The present study also suggests that in bovine male lymphocytes the X and the Y chromosomes initiate DNA synthesis at the beginning of the S period (Figure 4). This observation is in agreement with the findings in human cells by Kikuchi and Sandberg (1964) and Cave (1966). On the other hand, late initiation of DNA synthesis in the Y chromosome was reported by Schneider and Reike (1967) in the opossum, Taylor (1960) and Hsu (1964) in the Chinese hamster; Gartler and Burt (1964) in cattle; Bianchi and De Bianchi (1965), Sofuni and Sandberg (1967) and Takagi and Sandberg (1968a,b) in man.

The cause of conflict that exists in the literature regarding the pattern of initiation of DNA replication in mammalian sex chromosomes is difficult to determine. Mukherjee *et al*. (1968) pointed out that in many of the experiments which gave results suggesting that the 'late' replicating X and Y chromosomes were correspondingly late in initiating DNA synthesis, a chemical block such as FUdR (Hsu, 1964; Hsu and Lockhart, 1964; and Priest _et al._, 1967) or aminopterin (Petersen, 1964) had been used to synchronize cells at the beginning of the S period. The FUdR block was shown by Till _et al._ (1963) to alter the normal metabolism of L-strain mouse cells and to cause unbalanced RNA and protein synthesis during the time DNA synthesis was inhibited. These investigators also observed that the S period following FUdR treatment was shortened significantly. The FUdR or aminopterin treatment inhibits thymidylic acid synthesis and, therefore, DNA synthesis. However, if a pool of soluble thymidine derivatives is available in the cell or in the medium, then DNA synthesis may continue until the pool is exhausted. The presence of such a pool was reported to exist in calls of _Tetrahymena pyriformis_ (Stone _et al._, 1965) and in grasshopper neuroblasts (Leach, 1964) and may also exist in mammalian cells. Therefore, when the cells are treated with FUdR or aminopterin, DNA synthesis may continue for a short period of time until the pool is used up. If the time of exhaustion of the pool coincides with the interval when the rate of DNA synthesis in one X chromosome is lowest or temporarily interrupted, and with the time of pulse labeling with H^3TdR, one would expect very little or no labeling of one X chromosome. This situation may, therefore, be misinterpreted as delayed initiation of DNA synthesis in one X chromosome. However, before this explanation can be accepted completely, the existence of a pool of thymidine precursors in mammalian cells will have to be established.

The results of the FUdR experiments mentioned above are also at variance with a similar study in which repeated autoradiographs were made of a single cell of the Chinese hamster that was pulse labeled with H^3TdR in the first few minutes after an FUdR block (Stubblefield, 1965). After the separate autoradiographs were combined into a single photograph, it became clear that the Y chromosome and the 'late' replicating long arm of the X chromosome did indeed incorporate a significant amount of isotope during the early S period and were not late in initiating DNA synthesis.

Takagi and Sandberg (1968b) used a modification of the continuous labeling method of Mukherjee and Sinha (1965) that was designed to study the initiation of DNA replication in mammalian cells in culture. They observed that one X chromosome in the human female and the Y chromosome in the male initiate replication later than all other chromosomes in their respective complements. This contradicted the reports by Mukherjee and his co-workers (1965, 1966 and 1967). However, in the human complement the X and Y chromosomes cannot be identified unequivocally as they can be identified in the cow complement used by Mukherjee and co-workers. Furthermore, the timing of H^3TdR labeling and colchicine treatment in these experiments was different and may also be partly responsible for the difference in results.

The relative rates of DNA synthesis in individual chromosomes of the bovine male and female throughout the S period were estimated by determining their average grain densities in different parts of the S period. The validity of this method has been established by Hunt and Foote (1967) who showed that there was a high correlation between the number of grains counted in autoradiographs and the disintegrations per minute in a liquid scintillation counter in samples of rabbit spermatozoa labeled with H^3TdR. In addition, they found that on the average only one grain resulted from every 5.8 disintegrations.

In the male bovine experiment the graph of the average grain densities on the X and Y chromosomes suggest that the rate of DNA synthesis gradually increases from the beginning of the S period to about the mid S period and then decelerates towards the end of the S period (Figure 4). These results, however, should be taken with caution since the average grain densities varied only between 1 and 5 grains. The graph of the cumulative integrals of the X chromosome of the male (Figure 5) was approximately a straight line which suggests that the rate of DNA synthesis of the X chromosome is constant throughout most of the S period. There is, however, an indication of the presence of a short period of acceleration in DNA synthesis at the beginning of the S period and deceleration near the end of the S period. The graph of the average grain densities of the Y chromosome (Figure 4) suggests that the rates of DNA synthesis are relatively low in the first half of the S period and accelerate only slightly in this period.

In the second half of the S period there is a burst in the rate of DNA synthesis. The slopes of the graph of the cumulative integrals of the Y chromosome (Figure 5) are in agreement with this description.

The study of the rates of DNA synthesis in the female experiment required a slightly different approach than the one used in the male experiment because the heterochromatic and euchromatic X chromosomes could not be distinguished from one another morphologically. The autoradiographs of some female bovine lymphocytes labeled in the late S period were found to have one X chromosome more heavily labeled then the other X chromosome and the autosomes. An autoradiograph of such a cell can be seen in Plate 1 and the frequencies of such cells in different harvests are listed in Table III. This heavily labeled X chromosome must synthesize DNA at a higher rate than the other X chromosome and autosomes during this interval. This chromosome is considered to be the heterochromatic X chromosome which forms the Barr body in interphase nuclei. On the other hand, some metaphase cells labeled in the early S period had an unlabeled or lightly labeled X chromosome, the rest of the complement being heavily labeled (Plate 2 and Table III). This X chromosome considered to be the heterochromatic X chromosome must have been replicating at a rate lower than the rate of replication in the rest of the complement. In most metaphase cells labeled during the mid S period and in some cells labeled in the early and late S periods both X chromosomes were labeled as intensely as the autosomes (Plate 3 and Table III). The euchromatic X chromosome, the two longest autosomes and the other autosomes do not show any pattern of variation in labeling. Instead, the labeling of these chromosomes suggest that they incorporate H^3TdR at a fairly constant rate throughout the S period. Since the two X chromosomes are of equal size they must synthesize an equal amount of DNA during the S period. Therefore, the X chromosome that replicates at a higher rate in the late S period must be the same X chromosome that synthesizes at a lower rate in the early S period so that a balance in the amount of DNA synthesized by each of the two X chromosomes can be attained. The rate of replication of this asynchronously replicating X chromosome accelerates in the first half of the S period so that by mid S period its rate of replication equals the rate of replication of the rest of the complement. This is the 'switch-over' point in the rate of replication of the two X chromosomes. The acceleration continues so that by the late S period the heterochromatic X chromosome is replicating faster than the rest of the complement.

The visual patterns in labeling mentioned above were supported by the results of a chi-square test of the differences in grain densities between the two X chromosomes and between the two longest autosomes in a complement. On the basis of this analysis, the X chromosome pairs and the two longest autosomes were classified into categories depending on whether they had low (L category), similar (S category) or significantly different (D category) grain densities within a pair (Figures 7 and 8).

An attempt was then made to estimate the average grain densities on individual chromosomes, using a method that was designed to account for the fact that the heterochromatic and euchromatic X chromosomes could not be individually identified morphologically but could be distinguished from each other when their grain densities were significantly different. This method gives an estimate of the maximum possible differences in the relative rates of replication of the two X chromosomes and the two longest autosomes (this refers to the uncorrected average grain densities) and a corrected estimate of the relative rates of replication (this refers to the corrected average grain densities).

The above method of analysis gave results that were consistent with what one could generally predict from a visual analysis of the autoradiographs. The advantage in using this method however, is that the quantitative aspects of DNA replication can be studied.

The results of the analysis of the average grain densities strongly suggests that the two longest autosomes and one X chromosome in the bovine female complement synthesize DNA at a constant rate throughout most of the S period. This was suggested by the graphs of the uncorrected and corrected average grain densities on such chromosomes. These average grain densities were relatively constant throughout the S period (Figures 6 and 9). Furthermore, the slopes of the graphs of the cumulative integrals of each of these chromosomes

(Figures 10 and 11) were very similar and constant throughout the S period. Since these chromosomes are thought to be composed mostly of euchromatin, the results suggest that a constant rate of replication may be a property of all euchromatic chromosomes.

One X chromosome in the bovine female complement was found to replicate at a rate less than half the rate estimated for the other X chromosome and the two longest autosomes during the first few hours at the beginning of the S period (Figures 6 and 9). The rate of this slowly replicating X chromosome gradually accelerates however, and becomes equal to the rate of replication maintained by the other X chromosome and the autosomes in the mid S period and finally becomes at least twice as high as the other X chromosome and autosomes in the late S period. The cumulative integral graphs of this asynchronously replicating X chromosome also confirm this observation (Figure 10). Since this X chromosome is assumed to be the heterochromatic X chromosome, it can be generalized that heterochromatic chromosomes or chromosome regions begin replication at low rates and gradually accelerate in their rates of replication as the S period progresses.

The experimental design and methods used in this study were very similar to the ones used by Takagi and Sandberg (1968a) in a study on the rates of chromosome replication in normal human male complements and in cells from an individual with an X/iso-X sex chromosome constitution. However, unlike the experiments reported here, they treated the cells with 0.05 per cent colchicine one hour before harvest in order to collect more cells at metaphase. They examined 100 metaphase cells in each of 10 harvests made at intervals covering the first 11.5 hours after pulse and determined the average grain densities on certain pairs of autosomes that could be identified morphologically, on the iso-X and Y chromosomes and on the entire complement. The graphs of the average grain densities in the study by Takagi and Sandberg (1968a) suggest that DNA synthesis in the entire complement and on the pairs of autosomes began at low rates, accelerated slowly in the early S period, reached their maximum in the mid S period and decelerated rapidly in the late S period. In the first few hours of the S period, the Y chromosome did not replicate at all, or else replicated at very low rates. The rate then slowly accelerated and reached a maximum about 2 hours before the end of the S period and then decelerated. The iso-X chromosome was found not to replicate in the first 2 hours of the S period but accelerated to a maximum rate about 1 or 2 hours before the end of the S period. These results suggested that both euchromatic chromosomes (the autosomes) and heterochromatic chromosomes (the iso-X and Y chromosomes) gradually accelerated in rates of DNA synthesis as the S period progressed and then decelerated in the late S period. In this thesis the average grain density graphs of the two longest autosomes and the early replicating X chromosome of the bovine female suggested that these euchromatic chromosomes have a fairly constant rate of replication throughout the S period. An exception to this, which will be discussed further, is the average grain density graph of the X chromosome studied in the bovine male which suggested that this X chromosome gradually accelerated in the rate of DNA synthesis up to the mid S period and then decelerated.

On the other hand, the cumulative integral graphs of the various pairs of autosomes studied by Takagi and Sandberg (1968a) approached the form of a straight line throughout most of the S period. This suggested that the rate of DNA synthesis on these euchromatic chromosomes was fairly constant throughout the S period. This conclusion agreed with the conclusion arrived at with the cumulative integral graphs of the two longest autosomes and the early replicating X chromosome in the bovine female and the X chromosome in the bovine male. The cumulative integral graphs of the heterochromatic Y and iso-X chromosomes in humans (Takagi and Sandberg, 1968a) and the heterochromatic X and Y chromosomes in bovines (this study) suggested that these chromosomes replicated throughout the S period with a gradual acceleration in the rate of replication towards the end of the S period.

Conclusions on the rates of replication arrived at using the average grain density graphs of the euchromatic chromosomes in the study by Takagi and Sandberg (1968a) and the X chromosome of the bovine male in this study appear to be in disagreement with the conclusions arrived at using the cumulative integral graphs of the same chromosomes. This may be due to the fact that the average grain densities were always less than 10 grains per chromosome which does not represent a wide range in the average grain densities.

The fact that colchicine was used in the experiment of Takagi and Sandberg (1968a) but not in the present investigation may also explain why conclusions differed. The colchicine treatment arrests cells from as broad a period of the cell cycle as the period of treatment (1 hour in the reported study). Therefore, average grain densities would be estimates of the relative rates of DNA synthesis over whole hour intervals of the S period. This mixing at metaphase of cells that were in a 1 hour span of the cell cycle during the pulse could give a mixture of unlabeled metaphases that were in the G1 period and labeled metaphases that were in the initial hours of the S period. Similarly, cells labeled in the terminal hour of the S period would be mixed at metaphase with cells that were in the G2 period. Therefore, the unlabeled figures in these harvests would tend to lower the average grain densities and give an estimate of the rate of replication that was too low for the terminal and initial stages of the S period.

The major speculation in this thesis is a new hypothesis on gene action and dosage compensation in the X chromosomes of mammals, suggested by the experimental results. The hypothesis states that regions of the heterochromatic X chromosome in the female show a response to the stimulus for RNA synthesis that is correlated with their time of DNA synthesis. Furthermore, the amount of RNA synthesized by homologous X-linked genes in the female is equivalent to the amount synthesized by the same gene on the single X chromosome in the male.

In the experiment using bovine female lymphocytes, it was found that approximately one-quarter of the DNA of the heterochromatic X chromosome is replicated in the first half of the S period. It is postulated that these early replicating regions of the heterochromatic X chromosome do respond to the stimulus for RNA synthesis. Furthermore, the experimental results showed that approximately three-quarters of the DNA of the heterochromatic X chromosome was replicated in the second half of the S period. It is postulated that most of the genes in these late replicating regions of the heterochromatic X chromosome do not respond, or respond very little, to the stimulus for RNA synthesis. Furthermore, it is postulated that the total amount of RNA synthesized by homologous genes in the female equals the amount synthesized by the same gene on the single X chromosome in the male. This hypothesis does not specify the proportions of RNA that is synthesized by each of the two homologous genes. The gene on the heterochromatic X chromosome may synthesize as much RNA as the homologous gene on the euchromatic X chromosome or else a smaller proportion. Detailed studies would have to clarify this point.

A question raised by this hypothesis concerns the relationship that might exist between late DNA synthesis and sensitivity to the stimulus for RNA synthesis in euchromatic chromosomes. It may be either that no relationship exists, or that these late replicating regions of basically euchromatic chromosomes are actually heterochromatic segments and do show a correlation in DNA and RNA synthetic behavior.

If a gene is located in the genetically inactive and late replicating portion of the heterochromatic X chromosome and if the maternal and paternal X chromosomes in the female have an equal chance of being the heterochromatic X chromosome in each cell, then in females heterozygous for a normal and mutant allele of such a gene, one could expect a mosaic female with two cell types. One cell type would have the phenotype of the normal allele and the other cell type would have the phenotype of the mutant allele. This explains the observation of two such cell types in heterozygous females with the genes for glucose-6-phosphate dehydrogenase deficiency (Davidson et al., 1963; Beutler et al., 1962), Hurler's syndrome (Danes and Bearn, 1967), the Xg^a blood group (MacDiarmid et al., 1967), and hypoxanthine-guanine phosphoribosyl transferase deficiency (Migeon et al., 1968). Essentially, this section of the hypothesis dealing with the genes in the late replicating regions of the heterochromatic X chromosome is analogous to the Lyon hypothesis which deals with all the genes in the X chromosomes.

On the other hand, if a gene is located in the genetically active and early replicating regions of the heterochromatic X chromosome, then the genetic effects of RNA synthesized by the genes on the heterochromatic X chromosome may eventually interact with the genetic effects of RNA synthesized by the homologous gene on the euchromatic X chromosome or by other active genes on either X chromosome. Genetic interaction involving X-linked genes in heterozygous females was suggested in a hypothesis by Gruneberg (1967b) to explain why the hair structure was abnormal throughout the coat of female mice heterozygous for each of the X-linked genes tabby (Ta), striated (Str) and brindled (Mo^{br}), (Gruneberg, 1966b). According to the Lyon hypothesis these mice should have patches with the normal type of hair structure and patches with the mutant type of hair structure. Furthermore, Gruneberg (1967b) observed genetic interaction in doubly heterozygous mice with the genotypes Ta + / + Str, Ta + / + Mo^{br} and Ta Mo^{br} / + +.

Another source of evidence for the concept that the heterochromatic X chromosome is not always completely genetically inactive was obtained from a study by Ferguson-Smith (1965). He correlated karyotypes and phenotypes of patients who had gonadal dysgenesis along with some features of Turner's syndrome. An important observation was that individuals who were X/iso-X for the long arm, and therefore monosomic for the short arm, had more features of Turner's syndrome than individuals who were X/iso-X for the short arm, and therefore monosomic for the long arm. This information was related to the findings in previous publications showing that iso-X chromosomes were always the late replicating X chromosome of the complement, and therefore, the heterochromatic X chromosome (Grumbach et al., 1963; Miller et al., 1963; Muldal et al., 1963; Mukherjee et al., 1966). In order to explain the differences observed among the patients, Ferguson-Smith suggested that there are more genes on the iso-X chromosome for the short arm that are not suppressed but instead are genetically active in preventing Turner's syndrome than there are in the iso-X chromosome for the long arm. In other words, there are genes in these abnormal X chromosomes, especially in the iso-X chromosome for the short arm and presumably in the short arms of normal X chromosomes that remain genetically active in preventing various features of Turner's syndrome, even when the genes are found in the heterochromatic state.

VI. CONCLUSIONS

1. The two X chromosomes of the bovine female complement and the single X chromosome of the male initiate replication at the beginning of the S period along with the rest of the chromosomes in the complement. The Y chromosome may also commence replication at the beginning of the S period.

2. Euchromatic chromosomes, such as the early replicating X chromosome of the female, the single X chromosome of the male and the two longest autosomes in the female, replicate at a constant rate throughout the S period with a short period of acceleration in the beginning and deceleration at the end of the S period.

3. Heterochromatic chromosomes, for example the late replicating X chromosome of the female and the Y chromosome of the male, begin replication simultaneously with the other chromosomes but at low rates and accelerate gradually as the S period progresses and finally replicate more rapidly than the rest of the chromosome complement in the late S period.

4. The average duration of the G1, S and G2 periods of female bovine lymphocytes was found to be 2, 8 and 2 hours, respectively, and the total cell cycle had an average duration of 12 hours.

5. It is postulated that the heterochromatic X chromosome probably is not totally genetically inactive at all loci. On the

heterochromatic X chromosome enough RNA may be synthesized by the genes that replicate in the early S period, to enable genetic interaction with homologous genes or other active genes in female cells, as proposed by Gruneberg (1967b). On the other hand, genes in the late replicating regions of the heterochromatic X chromosome may be ineffective in synthesizing RNA and, therefore, would fit the postulates of the hypothesis proposed by Lyon (1962).

VII. APPENDIX

The literature in this field is extensive and sometimes conflicting. Some of the confusion may be due to technical artifacts. However, it is reasonable to suppose that some of the discrepancies in the literature are due to real biological differences among species, tissues or physiological conditions. This review was made to determine which of these factors may be involved and to provide background information so that the analysis and interpretation of the experimental results of this thesis may be understood in relation to other replication studies.

The review was organized phylogenetically with comments on the karyotype, cell cycle, and replication patterns of chromosomes, particularly the sex chromosomes. Each karyotype was described briefly so that the reliability of identification of the sex chromosomes from the rest of the complement could be known. The cell cycle studies were reviewed because it is difficult to study the rates and patterns of chromosome replication throughout the S period unless the time of DNA synthesis in relation to the total cell cycle is known.

Order: RODENTIA Family: CRICETIDEA

Cricetulus griseus (Chinese hamster)

The Chinese hamster has a chromosome complement of 2n = 22 chromosomes (Hsu and Benirschke, 1967, Folio No. 13). The X chromosome may not always be distinguished from chromosome pair number 4. The Y chromosome and 4 autosomal pairs can be clearly distinguished.

The minimum, average and maximum estimates of the duration of the periods of the cell cycle are: G1 period = 2, 4 and 16 hours; and G2 period = 1, 3 and 5 hours. The S period varies from 6 to 8.5 hours. The total cell cycle ranges from 10 to 24 hours but averages 12 hours in duration (Table IV).

In cultured cells from the male, during the late S period, the long arm of the X chromosome and the entire Y chromosome replicate at higher rates than the short arm of the X chromosome and the autosomes.(Taylor, 1960; Hsu, 1964; and Utakoji and Hsu, 1965). This is the well known late replication pattern. On the other hand, if the rate of incorporation of H^3TdR of the sex chromosomes is the same as the rate found among the autosomes throughout the S period then this is referred to as the early replication pattern. According to Hsu (1964), the late replicating long arm of the X chromosome and the entire Y chromosome begin replication later in the S period than all the other chromosomes in the complement. In cells from the female one X chromosome replicates in the same way as the single X chromosome of the male. The other X chromosome of the female replicates along its entire length in the second half of the S period and either does not replicate at all, or only slightly, in the early S period. According to the estimates of Hsu (1964) and Taylor (1960) the late replicating portions of the X and Y chromosomes replicate only during the last 25 to 35 per cent of the S period. The report by Stubblefield (1965) does not confirm this, but rather, strongly suggests that DNA synthesis does occur in these late replicating chromosomes in the very early S period but at rates lower then in the rest of the complement.

Some studies indicate that there are late replicating regions of the autosomes which also have low rates of DNA synthesis during the early S period (Hsu, 1964; Stubblefield, 1965). Labeling patterns of homologous autosomes in the early S period may be similar or different at particular loci (Stubblefield, 1965). The patterns of chromosome replication are relatively constant in different somatic tissues of the animal (Pflueger and Yunis, 1966; Martin, 1966).

Order: RODENTIA Family: CRICETIDAE

Mesocricetus auratus (Syrian or "golden" hamster)

The Syrian hamster has a chromosome complement of 2n = 44 chromosomes (Hsu and Benirschke, 1967, Folio No. 14). The X chromosome is the largest chromosome in the complement and, therefore, can be easily identified. The Y chromosome may not always be distinguished from some of the large submetacentric autosomes. The autosomes can be classified into several morphological groups according to their arm ratios and lengths. The X chromosome is a duplicate type chromosome because its length is 10 per cent of the total haploid complement, whereas in most other mammals this value is about 5 per cent.

No analysis of the cell cycle has yet been reported for this species.

The replication patterns of the sex chromosomes in the late S period, determined by continuous end labeling with H^3TdR, are found to be essentially the same in short term cultures established from 15 day fetuses (Galton and Holt, 1964) and from 7.5 to 9.5 day-old embryos and adult skin fibroblasts, but are different in 3 day-old, eight-cell embryos before implantation (Hill and Yunis, 1967). Except in embryos before implanatation, the long arm of one X chromosome in female cells and the long arm of the X chromosome in male cells are late replicating. The entire second X chromosome in female cells and the whole Y chromosome in male cells also replicate at a relatively high rate during the late S period. In cells of female embryos before implantation only the long arms of both X chromosomes are late replicating, and the short arms are early replicating and in cells of male embryos the Y chromosome is early replicating (Hill and Yunis, 1967).

Order: RODENTIA Family: MURIDAE

Mus musculus (Mouse)

The mouse has a chromosome complement of 2n = 40 chromosomes. All the chromosomes in the complement are acrocentric with only a slight variation in length making it morphologically impossible to identify individual pairs of chromosomes (Hsu and Benirschke, 1967, Folio No. 17). Autoradiography is helpful in identifying one X chromosome in the female and the Y chromosome in the male.

The estimated minimum, average and maximum duration of the periods of the cell cycle are: G1 period = 2, 3, and 4.5 hours; S period = 6.5, 7.5 and 14 hours; G2 period = 1, 3 and 8 hours and the total cell cycle = 9, 15 and 20 hours (Table V).

A late replicating chromosome, which is among the 5 or 6 largest pairs of chromosomes in the complement is found in cells from the female (Galton and Holt, 1965). No late replicating chromosome of the same size is found in males. These findings suggest that this late replicating chromosome is an X chromosome. The Y chromosome, which is one of the smallest chromosomes in the complement, is presumed to be the heavily labeled chromosome in cultured embryonic cells labeled during the late S period.

Evans *et al.* (1965) studied the chromosome replication patterns in cells from female mice *in vivo* with an X chromosome - autosome translocation (Cattanach's translocation). Since this translocated X chromosome is the longest chromosome in the complement it could be easily identified. In 50 per cent of the cells labeled in the late S period the translocated X chromosome showed the late replication pattern while in the other 50 per cent of the cells a presumptive X chromosome of normal morphology showed the late replication pattern. In metaphase cells labeled in the early S period one X chromosome, either the normal X chromosome or the translocated X chromosome, was unlabeled or lightly labeled. These observations suggest that one X chromosome in the female replicates at a relatively low rate during early S period and at a rate relatively higher than the rest of the complement during the late S period and that each X chromosome has an equal chance of being this asynchronously replicating chromosome.

Order: RODENTIA Family: MURIDAE

Rattus norvegicus (Rat)

The rat has a chromosome complement of 2n = 42 chromosomes. There are 22 metacentric, submetacentric or subtelocentric autosomes and 18 acrocentric autosomes (Hsu and Benirschke, 1967, Folio No. 18; Hungerford and Nowell, 1963; and Chang et al., 1965). Except for the two largest pairs of autosomes, individual autosomal pairs cannot be definitely identified. The X chromosome may or may not have short arms and can usually be distinguished from autosome pair 3 by the absence of satellites and by a relatively smaller size. The Y chromosome is the smallest positively heteropyknotic element in some rat strains, but in other strains it cannot be distinguished from medium-sized acrocentric autosomes and is isopyknotic.

The estimates of the minimum, average and maximum duration of the periods of the cell cycle are: G1 period = 1, 3 and 6 hours; S period = 6, 8 and 9 hours; G2 period = 0.7, 3 and 7 hours and the total cell cycle = 10, 12 and 15 hours (Table VI). Bianchi and De Bianchi (1966a) reported that the G1 period, S period and total cell cycle have durations of 15, 21 and 35 hours, respectively. This is an unusually long estimate in comparison with other reports on the cell cycle in the rat and other mammals.

Using a pulse labeling and serial harvesting technique, Chang *et al*. (1965) observed that in the first 2 hours of the S period one X chromosome in the female does not replicate at all or replicates only very little, but that in the mid S period this same X chromosome replicates at a rate similar to the rest of the complement and in the late S period it replicates more than the other X chromosome and the autosomes. Bianchi (1966b), however, did not find a late replicating X chromosome in bone marrow cells *in vivo*. He also reported that both X chromosomes begin replication early in the S period and finish before other chromosomes in the complement. Using short term cultures of female fetal tissues Bianchi and De Bianchi (1966a) observed that one X chromosome of the female begins and terminates replication in the late S period. The Y chromosome was also found to begin and terminate replication later than the other chromosomes (Bianchi and De Bianchi, 1966a; and Bianchi, 1966b).

Order: RODENTIA Family: MURIDAE

Rattus (Mastomys) natalensis (African mouse, mastomys)

The complement of mastomys consists of 2n = 36 chromosomes. The submetacentric X chromosome is the largest chromosome of the complement. The Y chromosome is among the longest subtelocentric chromosomes in the complement (Huang, 1968; Hsu and Benirschke, Folio No. 71, 1968).

The estimates of duration of the periods of the cell cycle were: G1 period = 2 to 4 hours; S period = 9 hours; G2 period = 2 to 4 hours; and the total cell cycle = 14 to 16 hours (Huang, 1968).

In female cells one whole X chromosome and the long arm of the other X chromosome are late replicating. In male cells the long arm of the X chromosome and the entire presumptive Y chromosome are late replicating (Huang, 1968). These late replicating regions were also reported not to replicate in the early S period or to replicate very little. Similar results were obtained using embryonic cells and bone marrow cells in vitro and bone marrow cells in vivo.

Order: RODENTIA Family: ERETHIZONTIDAE

Erethizon dorsatum dorsatum (North American porcupine)

The North American porcupine has a chromosome complement of 2n = 42 (Benirschke, 1968; Hsu and Benirschke, Folio No. 72, 1968). The X chromosome is submetacentric and the largest chromosome in the complement and consists of about 12 per cent of the length of a haploid set of chromosomes. The Y chromosome is submetacentric, about the same size as some of the larger autosomes and consists of about 6 per cent of the length of a haploid set of chromosomes.

The cell cycle has not been analyzed in this species.

In the male porcupine the long arm of the Y chromosome and the distal regions of both arms of the X chromosome are late replicating (Benirschke, 1968).

Order: RODENTIA Family: CHINCHILLIDAE

Chinchilla laniger (Chinchilla)

The chromosome complement of the Chinchilla is 2n = 64 chromosomes. The autosomes are metacentric or submetacentric in structure and individual autosomal pairs cannot readily be distinguished (Hsu and Benirschke, 1967, Folio No. 19; Galton et al., 1965). The metacentric X chromosome may be easily identified since it is the largest element in the complement consisting of approximately 9 per cent of the length of the haploid set of autosomes. The Y chromosome is not easily identified but it is one of the smallest chromosomes in the complement and is submetacentric or acrocentric in structure.

No cell cycle analysis has been reported for this species.

In a culture of kidney cells Galton et al. (1965) observed that one X chromosome in female cells and the X chromosome of male cells are late replicating in their short arms and distal portions of the long arms. The regions of the long arm adjoining the centromere in each chromosome are early replicating. The entire second X chromosome in female cells is also late replicating. The presumptive Y chromosome in the male replicates at a higher rate than other chromosomes in the late S period and is one of the last chromosomes in the complement to finish replication.

Order: CARNIVORA Family: CANIDAE

Canis familiaris (Dog)

The dog chromosome complement (2n = 78) is particularly useful in cytogenetic studies because the submetacentric X chromosomes are easily distinguished from the 76 acrocentric and telocentric autosomes (Hsu and Benirschke, 1967, Folio No.20). The metacentric Y chromosome, occasionally acrocentric, is one of the smallest elements in the complement. The only autosomal pair that can be definitely identified is the longest pair in the complement.

The cell cycle was analyzed using bone marrow cells *in vitro* with the pulse labeling and serial harvest method (Brown *et al*., 1966). The average duration of the periods of the cell cycle were found to be: G1 period = 1.5 hours; S period = 8 hours; G2 period = 4.5 hours and the total cell cycle = 12 to 14 hours. The shortest time for the cell cycle was estimated from the observation that the first cells with single-chromatid labeling appeared at 12 to 14 hours. These cells would have passed through two G2 period. Subtracting one G2 period, the briefest cell cycle is found to be 10.5 hours.

Replication rates of the chromosomes increase in the early S period in female bone marrow *in vitro*, except for one X chromosome which has a relatively low rate. This slow starting X chromosome presumably is the X chromosome which replicates at a high rate in the late S period and finishes late in the S period in bone marrow *in vivo* (Fraccaro *et al*., 1964) and *in vitro* (Brown *et al*., 1966) and in lymphocytes *in vitro* (Fraccaro *et al*., 1964). The single X chromosome in male cells shows an early replication pattern and the Y chromosome shows a late replication pattern (Brown *et al*., 1966).

Order: ARTIODACTYLA Family: BOVIDAE

Bos taurus (Cattle)

Bos taurus has a chromosome complement of 2n = 60 chromosomes. Like the chromosome complement of the dog, individual pairs of autosomes are only slightly different in size and are acrocentric in structure (see Plates 1 and 6). Both the X and Y chromosomes are submetacentric, but the X chromosome is one of the largest chromosomes in the complement and the Y chromosome is one of the smallest (Sasaki and Makino, 1962; Hsu and Benirschke, 1967, Folio No. 44).

Estimates of the average duration of the G1 period = 2 hours, S period = 8 hours, G2 period = 3 hours and the total cell cycle = 12 hours.

In female bovine lymphocytes in vitro one X chromosome is late replicating. The short arms of both X chromosomes terminate replication slightly late than the long arms as shown by continuous labeling with H^3TdR at the end of the S period (Mukherjee and Sinha, 1963; Evans, 1965). The results obtained from pulse labeling and serial harvest experiments agree with the above conclusions and demonstrate further that both X chromosomes of the female continue replication throughout the S period with variation in rates of replication (Gartler and Burt, 1964; Wright, this thesis). Both X chromosomes begin replication in the very early S period simultaneously or only slightly out of phase (Gartler and Burt, 1964) and even before some of the autosomes begin to replicate as shown by continuous labeling with H^3TdR during the early S period (Mukherjee _et al_., 1967). The Y chromosome begins replication about 2 hours after the initiation of the S period and finishes shortly after the X chromosome and autosomes (Gartler and Burt, 1964). The results of this thesis suggest that the Y chromosome initiates replication in the early S period and increases in the rate of replication as the S period progresses.

Order: PRIMATES Family: HOMINIDAE

Homo sapiens (Man)

In the human chromosome complement of 2n = 46 chromosomes it is difficult to distinguish the submetacentric X chromosome from the submetacentric autosomes of the C group (6-12). The acrocentric Y chromosome, which is morphologically similar to the autosomes of group G (21-22) cannot be identified in most of the metaphase figures (CHICAGO CONFERENCE, 1966; Hsu and Benirschke, 1967, Folio No. 50).

Variation exists among the estimates of the duration of the periods of the cell cycle. In fibroblast-like cell cultures the estimates of the minimum, average and maximum durations are: G1 period = 4, 7 and 10 hours; S period = 7, 8 and 14 hours; G2 period = 2, 5 and 8 hours and the total cell cycle = 18, 20 and 22 hours (Table VIII). In lymphocyte cell cultures the estimates are: G1 period = 4, 6 and 8 hours; S period = 7 to 20 hours; G2 period = 1.5, 3 and 10 hours and the total cell cycle = 17.7, 20 and 28 hours (Table IX).

In cells from the human female the presence of a late replicating X chromosome that is heteropyknotic in prophase and forms the Barr body in interphase, is well established (Tables VIII, IX and X). Reports vary as to whether the late replicating X chromosome is the last chromosome to end replication, but the conflict appears to deal with only an interval of a few minutes (Gilbert _et al_., 1965). In cells with more than two X chromosomes, all but one X chromosome show the late replication pattern. Abnormal X chromosomes in man such as iso-X chromosomes and ring X chromosomes are always found to show the late replication pattern. The pattern of replication of the late X chromosome in the early S period is controversial. Some reports state that this X chromosome does not replicate in the first third or half of the S period. Other reports state that the late X chromosome begins replication with the rest of the complement and continues to replicate throughout the S period. According to some reports the Y chromosome replicates very little or not at all in the early S period but shows the late replication pattern. But, other reports indicate that the Y chromosome does not show a significant late replication pattern.

VIII TABLES

TABLE I

GRAINS COUNTED ON THE X AND Y CHROMOSOMES OF BULL LYMPHOCYTES PULSE LABELED WITH H^3TdR AND HARVESTED AT DIFFERENT TIMES AFTER PULSE.

Cell No.	X	Y
Harvest Time : 3½ hours		
1-8	0	0
9	5	3
10	11	5
11	1	8
Average Grain Density	1.5	1.5
Harvest Time : 5 hours		
1-6	0	0
7	11	8
8	6	2
9	2	11
10	7	4
11	3	4
12	4	0
13	6	0
14	11	5
15	8	1
Average Grain Density	3.9	2.3
Harvest Time : 6½ hours		
1-4	0	0
5	2	0
6	4	1
7	8	1
8	2	2
9	3	2
10	7	0
11	1	3
12	10	0
13	4	0
14	7	3
15	5	0
16	11	2
17	1	0
18	14	3
19	0	4
20	10	2
21	16	4
22	2	0
23	2	0
24	6	0
25	6	0
26	2	0
Average Grain Density	4.8	1.0
Harvest Time : 8 hours		
1-19	0	0
20	8	2
21	4	0
22	12	0
23	5	2
24	4	0
25	1	0
26	5	1
27	2	1
28	12	0
29	1	0
30	8	0
31	5	0
32	6	1
33	5	0
34	4	2
35	5	0
36	1	0
37	3	0
38	5	0
39	11	0
40	3	0
41	4	2
42	3	2
43	11	4
44	2	0
45	2	0
46	9	5
47	5	0
48	3	0
49	10	2
50	2	0
51	4	0
52	3	0
53	4	0
54	2	0
55	5	0
56	5	0
57	0	2
58	6	1
59	3	0
Average Grain Density	3.3	0.5

TABLE I (CONTINUED)

Cell No.	X	Y
Harvest Time : 9 hours		
1-32	0	0
33	3	0
34	7	0
35	4	1
36	5	0
37	3	0
38	2	0
39	6	2
40	3	0
41	2	0
42	1	1
43	4	1
44	3	0
45	1	0
46	2	0
47	1	0
48	3	0
49	2	0
50	4	1
51	5	0
52	4	0
53	5	0
54	1	0
55	4	0
56	5	2
57	6	0
58	2	0
59	4	0
60	2	0
61	1	0
62	2	0
Average Grain Density	1.6	0.1
Harvest Time : 9½ hours		
1-14	0	0
15	5	0
16	7	0
17	1	0
18	8	4
19	3	0
20	3	0
21	12	6
Average Grain Density	1.9	0.5

Cell No.	X	Y
Harvest Time : 10½ hours		
1-20	0	0
21	1	2
22	5	0
23	3	0
24	1	0
25	4	0
26	1	0
27	7	4
28	16	3
Average Grain Density	1.4	0.3
Harvest Time : 11 hours		
1-18	0	0
19	6	1
20	8	0
21	2	0
22	1	0
23	2	0
24	4	0
25	8	2
26	2	2
27	2	2
Average Grain Density	1.3	0.3
Harvest Time ; 12½ hours		
1-34	0	0
35	6	3
36	3	0
37	12	6
38	2	0
39	3	2
Average Grain Density	0.7	0.3

TABLE II

GRAINS COUNTED ON THE X CHROMOSOMES AND TWO LONGEST AUTOSOMES IN METAPHASE CELLS OF COW LYMPHOCYTES PULSE LABELED WITH H^3TdR AND HARVESTED AT DIFFERENT TIMES AFTER PULSE. THE PAIRS OF X CHROMOSOMES AND TWO LONGEST AUTOSOMES ARE CLASSIFIED ON THE BASIS OF A CHI-SQUARE TEST AS HAVING GRAIN DENSITIES THAT ARE SIMILAR (S CATEGORY), DIFFERENT (D CATEGORY) OR TOO LOW FOR CHI-SQUARE TESTING (L CATEGORY). THE OTHER SYMBOLS MEAN: X-H, HEAVILY LABELED X CHROMOSOME; X-L, LIGHTLY LABELED X CHROMOSOME; A-H, HEAVILY LABELED AUTOSOME; A-L, LIGHTLY LABELED AUTOSOME.

Harvest Time : 1 hour 40 minutes

Cell No.	X-H	X-L	Category of pair	A-H	A-L	Category of pair
1	5	1	L	3	0	L
2	9	0	L	0	0	L
3	8	0	L	0	0	L
4	19	10	S	16	8	S
5	1	1	L	9	4	S
6	4	1	L	3	0	L
7	4	3	L	4	2	L
8	8	0	L	2	0	L
9	40	27	S	22	13	S
10	17	14	S	9	6	S
11	40	9	D	10	1	D
12	5	4	L	8	4	S
13	7	3	S	11	4	S
14	3	0	L	2	0	L
15	5	0	L	5	0	L
16	4	0	L	4	1	L
17	10	0	D	7	5	S
18	7	5	S	4	0	L
19	0	0	L	4	3	L
20	15	2	D	5	0	L
21	3	2	L	4	0	L
22	0	0	L	3	2	L
23	0	0	L	1	0	L
24	0	0	L	0	0	L
25	1	0	L	4	3	L
26	0	0	L	2	0	L
Average Grain Density	8	3		5	2	

TABLE II (CONTINUED)

Harvest Time : 2 hours

Cell No.	X-H	X-L	Category of pair	A-H	A-L	Category of pair
1	45	6	D	10	0	D
2	4	3	L	2	1	L
3	50	18	D	33	22	S
4	60	13	D	7	2	L
5	55	13	D	25	16	S
6	16	5	D	7	5	S
7	20	8	D	26	9	D
8	25	16	D	18	5	D
9	50	4	D	7	6	S
10	3	3	L	7	0	L
11	5	3	L	13	9	S
12	10	4	S	2	0	L
13	19	3	D	7	1	L
14	48	23	D	44	25	D
Average Grain Density	29	9		15	7	

TABLE II (CONTINUED)

Harvest Time : 3 hours

Cell No.	X-H	X-L	Category of pair	A-H	A-L	Category of pair
1	70	41	D	32	30	S
2	42	14	D	31	26	S
3	80	29	D	24	20	S
4	8	2	S	15	1	D
5	55	48	S	55	48	S
6	76	25	D	52	31	D
7	48	40	S	35	17	D
8	29	20	S	40	26	S
9	70	7	D	21	16	S
10	2	0	L	2	0	L
11	27	25	S	21	21	S
12	70	18	D	16	7	S
13	26	24	S	22	11	S
14	40	34	S	44	31	S
15	20	14	D	15	4	D
16	60	40	D	65	58	S
17	39	37	S	35	32	S
18	48	15	D	27	25	S
19	68	35	D	24	21	S
20	25	25	S	22	13	S
21	40	30	S	28	16	S
22	15	10	S	2	0	L
23	38	0	D	0	0	L
24	48	11	D	14	11	S
25	45	34	S	20	6	D
26	44	35	S	36	28	S
27	38	20	D	29	28	S
28	40	34	S	16	11	S
Average Grain Density	43	24		27	19	

TABLE II (CONTINUED)

Harvest Time : 4 hours

Cell No.	X-H	X-L	Category of pair	A-H	A-L	Category of pair
1	36	12	D	16	8	S
2	28	24	S	26	26	S
3	37	28	S	24	24	S
4	36	19	D	50	18	D
5	27	20	S	14	12	S
6	52	19	D	25	22	S
7	25	9	D	25	23	S
8	30	20	S	14	10	S
9	25	22	S	45	30	S
10	24	18	S	21	12	S
11	25	19	S	29	26	S
12	24	14	S	19	10	S
13	33	22	S	18	16	S
14	7	4	S	19	12	S
15	17	11	S	36	23	S
16	3	1	L	2	0	L
Average Grain Density	27	16		24	17	

TABLE II (CONTINUED)

Harvest Time : 5 hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	31	18	D	41	39	S
2	30	26	S	40	29	S
3	33	26	S	18	18	S
4	30	33	S	45	24	D
5	32	8	D	25	21	S
6	28	20	S	20	18	S
7	40	14	D	38	37	S
8	22	15	S	22	6	D
9	31	12	D	24	18	S
10	23	11	D	11	9	S
11	28	21	S	33	23	S
12	26	22	S	31	25	S
13	14	10	S	35	30	S
14	25	18	S	35	25	S
15	21	13	S	23	15	S
16	21	7	D	12	6	S
17	24	24	S	19	13	S
18	25	11	D	24	19	S
19	10	10	S	10	6	S
Average Grain Density	26	16		27	20	

TABLE II (CONTINUED)

Harvest Time : 6 hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	36	32	S	55	40	S
2	20	18	S	19	8	D
3	13	5	S	13	10	S
4	14	9	S	14	11	S
5	33	20	S	31	28	S
6	24	14	S	25	18	S
7	36	27	S	45	35	S
8	20	17	S	14	10	S
9	23	16	S	40	20	D
Average Grain Density	24	18		28	20	

TABLE II (CONTINUED)

Harvest Time : 7 hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	20	3	D	24	21	S
2	20	13	S	19	18	S
3	16	14	S	30	20	S
4	12	11	S	14	8	S
5	27	12	D	12	4	D
6	32	14	D	35	22	S
7	16	8	S	28	15	D
8	8	0	L	12	11	S
9	9	4	S	25	19	S
10	15	13	S	23	13	S
11	12	12	S	14	12	S
12	16	4	D	14	12	S
13	29	9	D	21	20	S
14	44	13	D	32	20	S
15	15	13	S	15	15	S
16	11	11	S	19	14	S
17	23	13	S	26	23	S
18	5	1	L	12	6	S
19	4	2	L	7	4	S
20	25	12	D	3	2	L
21	15	14	S	12	11	S
22	26	14	S	26	23	S
23	22	1	D	15	6	D
24	30	23	S	19	9	S
25	31	24	S	11	8	S
26	14	8	S	28	23	S
27	20	8	D	35	25	S
Average Grain Density	19	10		20	14	

TABLE II (CONTINUED)

Harvest Time : 8 hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	26	2	D	27	26	S
2	35	14	D	39	33	S
3	19	6	D	10	8	S
4	8	4	S	7	3	S
5	32	18	D	30	16	D
6	29	29	S	25	24	S
7	6	3	L	2	1	L
8	9	4	S	11	10	S
9	8	3	S	10	7	S
10	7	6	S	4	0	L
11	30	9	D	31	24	S
12	37	25	S	37	22	S
13	30	7	D	46	28	D
14	17	5	D	45	30	S
15	24	3	D	36	27	S
16	26	23	S	60	36	D
17	28	11	D	33	28	S
18	21	1	D	40	38	S
19	23	8	D	40	37	S
20	11	5	S	14	13	S
21	9	8	S	33	27	S
22	18	3	D	22	18	S
23	15	15	S	31	17	D
24	21	13	S	13	11	S
25	5	2	L	15	12	S
26	11	5	S	17	16	S
27	10	6	S	14	13	S
28	42	39	S	27	25	S
29	16	9	S	28	24	S
30	9	0	L	13	7	S
Average Grain Density	19	10		25	19	

TABLE II (CONTINUED)

Harvest Time : 9 hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	8	1	L	6	6	S
2	4	2	L	1	0	L
3	11	4	S	35	11	D
4	7	6	S	15	11	S
5	6	1	L	9	4	S
6	3	1	L	3	2	L
7	14	10	S	26	15	S
8	8	2	S	20	9	D
9	2	2	L	9	3	S
10	8	3	S	19	18	S
11	31	4	D	28	25	S
12	6	0	L	8	8	S
13	9	3	S	8	3	S
14	14	5	D	22	8	D
15	5	2	L	3	0	L
16	10	3	D	13	6	S
17	11	0	D	6	4	S
18	11	2	D	8	3	S
19	9	6	S	4	1	L
20	19	16	S	39	31	S
21	7	0	L	24	13	S
22	3	0	L	2	0	L
23	2	0	L	11	4	S
24	3	3	L	4	2	L
25	5	3	L	4	0	L
26	3	1	L	2	0	L
27	8	2	S	15	13	S
Average Grain Density	8	3		13	7	

TABLE II (CONTINUED)

Harvest Time : 10 hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	2	0	L	8	2	S
2	9	3	S	19	14	S
3	13	13	S	22	19	S
4	2	1	L	10	6	S
5	13	4	D	45	30	S
6	6	2	L	5	4	S
7	11	4	S	27	24	S
8	13	4	D	27	19	S
9	3	0	L	8	3	S
10	13	0	D	13	12	S
11	6	2	L	8	1	L
12	10	9	S	21	18	S
13	10	4	S	7	1	L
14	3	0	L	2	1	L
15	4	0	L	5	2	L
16	8	5	S	31	15	D
17	2	2	L	8	7	S
18	7	4	S	5	3	L
19	7	2	L	11	2	D
20	30	15	D	31	30	S
21	8	6	S	6	2	L
22	14	10	S	20	17	S
23	7	2	L	9	7	S
24	9	5	S	32	14	D
25	7	1	L	5	0	L
26	19	0	D	23	22	S
27	3	1	L	14	5	D
28	15	10	S	22	15	S
29	4	2	L	5	4	L
30	15	9	S	6	6	S
31	12	0	D	8	1	L
32	6	0	L	20	16	S
Average Grain Density	9	4		15	10	

TABLE II (CONTINUED)

Harvest Time : 11 hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	1	0	L	6	2	L
2	6	3	L	6	0	L
3	5	2	L	12	0	D
4	26	6	D	33	24	S
5	10	3	S	17	8	S
6	2	0	L	6	0	L
7	20	20	S	31	12	D
8	5	2	L	5	0	L
9	7	2	L	2	1	L
10	1	0	L	0	0	L
11	2	0	L	5	1	L
12	2	2	L	5	4	L
13	4	3	L	5	3	L
14	2	0	L	7	2	L
15	4	1	L	12	11	S
16	4	3	L	7	0	L
17	2	2	L	6	3	L
18	2	1	L	6	2	L
19	4	0	L	4	1	L
20	12	0	D	29	25	S
21	6	0	L	5	3	L
22	6	5	S	17	8	S
23	1	0	L	4	3	L
24	2	2	L	6	5	S
25	3	0	L	10	5	S
26	5	1	L	2	0	L
27	12	6	S	15	9	S
28	8	0	L	7	7	S
29	6	3	L	7	4	S
30	11	0	D	12	6	S
Average Grain Density	6	2		10	5	

TABLE II (CONTINUED)

Harvest Time : $11\frac{1}{2}$ hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	10	0	D	11	7	S
2	18	2	D	8	8	S
3	2	0	L	9	8	S
4	2	2	L	9	3	S
5	9	0	L	5	0	L
6	9	4	S	4	2	L
7	4	3	L	4	4	L
8	9	2	D	4	3	L
9	10	2	D	4	3	L
10	10	7	S	2	1	L
11	4	2	L	7	3	S
12	10	0	D	8	5	S
13	6	5	S	7	2	L
14	21	13	S	18	10	S
15	9	5	S	9	2	D
16	10	4	S	2	0	L
17	22	14	S	16	13	S
18	17	3	D	6	5	S
19	16	12	S	6	3	L
20	16	5	D	18	5	S
21	10	10	S	9	4	S
22	24	11	D	20	16	S
23	22	16	S	20	27	S
24	9	1	D	2	0	L
Average Grain Density	12	5		9	6	

TABLE II (CONTINUED)

Harvest Time : 12 hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	0	0	L	2	2	L
2	2	0	L	3	0	L
3	7	0	L	0	0	L
4	3	1	L	2	0	L
5	0	0	L	5	0	L
6	4	3	L	2	0	L
7	12	2	D	2	0	L
8	2	0	L	7	1	L
9	13	3	D	5	1	L
10	3	0	L	1	0	L
11	10	4	S	1	0	L
12	0	0	L	2	0	L
13	2	1	L	0	0	L
14	7	0	L	11	4	S
15	0	0	L	0	0	L
16	7	0	L	2	0	L
17	19	8	D	40	22	D
18	6	3	L	8	6	S
19	6	0	L	3	0	L
20	2	2	L	8	2	S
21	5	2	L	4	3	L
22	4	3	L	0	0	L
23	17	5	D	28	15	D
24	1	0	L	0	0	L
25	0	0	L	0	0	L
26	5	2	L	2	2	L
27	3	1	L	5	0	L
28	1	0	L	5	3	L
29	25	16	S	17	4	D
30	3	2	L	3	3	L
31	1	0	L	2	0	L
Average Grain Density	5	2		5	2	

TABLE II (CONTINUED)

Harvest Time : $12\frac{1}{2}$ hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	4	0	L	7	4	S
2	6	0	L	4	3	L
3	3	0	L	0	0	L
4	3	1	L	1	0	L
5	3	2	L	6	3	L
6	0	0	L	3	0	L
7	0	0	L	2	1	L
8	3	2	L	4	1	L
9	1	0	L	3	1	L
10	4	4	L	7	0	L
11	3	2	L	7	1	L
12	3	2	L	2	0	L
13	3	2	L	4	0	L
14	2	0	L	2	1	L
15	8	3	S	10	4	S
16	0	0	L	4	0	L
17	3	0	L	4	2	L
18	1	0	L	3	2	L
19	2	0	L	6	4	S
20	8	4	S	2	2	L
21	11	6	S	1	0	L
22	4	3	L	7	0	L
23	32	23	S	28	21	S
24	31	17	D	28	17	S
25	6	2	L	5	0	L
26	1	0	L	4	1	L
27	5	3	L	1	0	L
28	0	0	L	6	1	L
29	6	0	L	3	3	L
30	0	0	L	4	3	L
31	4	2	L	5	3	L
32	3	1	L	4	2	L
Average Grain Density	5	2		6	3	

TABLE II (CONTINUED)

Harvest Time : 13 hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	3	0	L	4	0	L
2	18	17	S	11	7	S
3	2	0	L	0	0	L
4	3	0	L	0	0	L
5	2	0	L	1	0	L
6	1	0	L	0	0	L
7	0	0	L	2	0	L
8	0	0	L	0	0	L
9	0	0	L	3	0	L
10	2	0	L	3	2	L
11	2	1	L	0	0	L
12	23	21	S	39	17	D
13	12	2	D	19	16	S
14	2	2	L	0	0	L
15	0	0	L	0	0	L
16	31	30	S	19	13	S
17	28	17	S	21	20	S
18	7	6	S	13	11	S
19	1	0	L	2	1	L
20	0	0	L	2	0	L
21	0	0	L	2	0	L
22	2	0	L	1	0	L
23	5	3	L	0	0	L
24	3	0	L	2	0	L
25	2	2	L	1	0	L
26	0	0	L	4	0	L
27	2	0	L	2	0	L
28	22	15	S	18	10	S
29	0	0	L	3	0	L
30	2	0	L	1	0	L
31	23	6	D	15	10	S
32	16	8	S	20	15	S
33	7	2	L	2	0	L
34	0	0	L	0	0	L
35	2	0	L	0	0	L
36	10	9	S	16	15	S
37	3	0	L	0	0	L
Average Grain Density	6	4		6	4	

TABLE II (CONTINUED)

Harvest Time : 14 hours

Cell No.	X-H	X-L	Category in pair	A-H	A-L	Category in pair
1	3	2	L	5	2	L
2	3	0	L	14	5	D
3	21	6	D	33	18	D
4	5	2	L	0	0	L
5	3	0	L	1	0	L
6	4	0	L	4	2	L
7	0	0	L	11	4	S
8	18	6	D	21	15	S
9	43	25	D	25	9	D
10	4	1	L	5	0	L
11	0	0	L	0	0	L
12	1	0	L	0	0	L
13	6	2	L	3	0	L
14	5	0	L	1	1	L
15	3	0	L	2	0	L
16	2	0	L	2	0	L
17	5	2	L	2	0	L
18	0	0	L	1	0	L
19	2	0	L	0	0	L
20	2	0	L	2	2	L
21	3	0	L	3	2	L
22	0	0	L	0	0	L
23	15	1	D	23	15	S
24	0	0	L	3	0	L
25	2	0	L	0	0	L
26	5	0	L	2	0	L
27	1	0	L	8	0	L
28	4	1	L	1	0	L
Average Grain Density	6	2		6	3	

TABLE III

AUTORADIOGRAPHIC LABELING PATTERNS OF 200 METAPHASE FIGURES IN EACH HARVEST AFTER PULSE LABELING WITH H^3TdR

Harvest Time Hours:Minutes	Labeled Metaphases					Unlabeled Metaphase Figures
	Both X chromosomes labeled as much as the autosomes	One X chromosome labeled more than the rest of the complement	One X chromosome labeled less than the rest of the complement	Total complement lightly labeled	Single chromatid labeling	
1:40	14	16	0	19	0	151
2	87	38	0	14	0	61
3	165	7	0	7	0	21
4	194	0	0	2	0	4
5	191	3	0	0	0	4
6	192	0	0	2	0	6
7	184	0	0	11	0	6
8	166	0	15	7	0	5
9	117	0	5	31	0	12
10	96	0	0	14	0	47
11	87	0	1	5	0	90
11:30	75	2	0	9	1	107
12	93	2	3	1	0	113
12:30	94	0	0	4	0	101
13	91	2	3	4	0	102
14	92	6	5	4	1	100
15	94	7	0	1	19	92
16	114	3	1	6	5	79
17	107	3	0	2	1	71
18	102	3	2	1	15	87
19	110	0	0	2	4	77
20	103	0	3	9	8	84
21	72	0	1	20	25	77
22	85	0	0	8	12	82
23	101	0	4	6	9	95
24	92	2	0	5	4	80
25	90	1	0	10	0	97
26	63	0	0	13	1	99

TABLE IV

CHINESE HAMSTER CELL CYCLE ANALYSIS

Tissue	Method	Duration of Cell Cycle Periods (Hours)				Reference
		G1 Period	S Period	G2 Period	Total	
Embryonic tissues *in vitro*	pulse labeling, serial harvest	1-2	8.5+	2.5+	12-14	Taylor, 1960
Ovary cells *in vitro*	cell synchronization by thymidine blockage, direct measurement of cell number, 3 media variations	5.5 8.7 16.3			13.3 16.5 24.1	Tobey *et al.*, 1967
Don cell line	continuous end labeling, FUdR synchronization and labeling of early S period		7.5+	2-3		Hsu, 1964
Don-C fibroblasts *in vitro*	Synchronization by selection of mitotic cells, analysis of incorporated tritiated precursors	2	8 (2 - 40 minute interruptions)	2	12+	Stubblefield *et al.*, 1967
Don lung cell line	various methods	2.95 3.84	5.94 5.65	2.48 (mitosis = 0.60) 1.91	11.97 12.00	
Bone marrow *in vivo*	pulse labeling, serial harvest		6-8	2	10 (variable)	Utakoji and Hsu, 1965
Tissues from 3 germinal layers *in vivo*	pulse labeling, serial harvest			1.5-5 (varies with tissue)		Pflueger and Yunis, 1966a, b
Minimum-Average-Maximum of Periods		2-4-16	6-8-8.5	1-3-5	10-12-24	

TABLE V

MOUSE CELL CYCLE ANALYSIS

Tissue	Method	Duration of Cell Cycle Periods (Hours)				Reference
		G1 Period	S Period	G2 Period	Total	
Fetal mouse tissues *in vivo*	pulse labeling, serial harvest		6.5-7		9-15	Cameron, 1964
Spleen, bone marrow, thymus - all *in vivo*	pulse labeling, serial harvest			1-2.5-4 2-3.5-6 2-5-8		Evans *et al.*, 1965
Lymphoblast strain 5178Y *in vitro*	pulse labeling, serial harvest; double time, flash labeling and harvest for proportion of cells in S period	2-2.5	7-7.5	2	11.5	Defendi and Manson, 1961
L-strain *in vitro*	pulse labeling, serial harvest, FUdR synchronization	4.5 (S period = 4 hours in the first cycle after FUdR treatment)	8	3 (mitosis = 0.5)	16	Till *et al.*, 1963
Minimum-Average-Maximum of Periods		2-4.5	6.5-7.5-14	1-3-8	9-15-20	

TABLE VI

RAT CELL CYCLE ANALYSIS

Tissue	Method	Duration of Cell Cycle Periods (Hours)				Reference
		G1 Period	S Period	G2 Period	Total	
Lung fibroblasts *in vitro*	pulse labeling, serial harvest	1-3-6	8-9	-3-5	-14-	Chang *et al*., 1965
Fetal tissue *in vitro*	pulse labeling, serial harvest	5-10-15	21	3-4-7	35+	Bianchi and De Bianchi, 1966a
			(considered a special case)			
Bone marrow *in vivo*	pulse labeling, serial harvest	-2-5	-7-	2-3-4	12-15	Bianchi, 1966b
Regenerating liver *in vivo*	biochemical and autoradiographic methods, H^3TdR labeling		8+			Looney *et al*., 1967
Lymphocytes	pulse labeling, serial harvest (cells were PHA stimulated then grown in diffusion chambers *in vivo*)	3-5	6	0.7	10-12	Johnson *et al*., 1967
Minimum-Average-Maximum of Periods		1-3-6	6-8-9	0.7-3-7	10-12-15	

TABLE VII

BOS TAURUS CELL CYCLE ANALYSIS

Tissue	Method	Duration of Cell Cycle Periods (Hours)				Reference
		G1 Period	S Period	G2 Period	Total	
Epithilial cell bine from calf liver	doubling time measurement (total) autoradiography (S and G2) cinematography (M)	16	8	6 (mitosis = 1)	31	Kuyper *et al.*, 1962
Calf kidney fibroblast cultures	pulse labeling, serial harvest		8+	1-3.5-4	12+	Gartler and Burt, 1964
Lymphocytes *in vitro*	pulse labeling, serial harvest	-2-4	8	1-2-4	10-12-14	Wright, 1968 (this thesis)
Minimum-Average-Maximum of Periods		-2- (16 extreme)	-8-	1-3-6	10-12-31	

HUMAN CELL CYCLE ANALYSIS

Tissue	Sex Chromosome Replication
Peritoneum XX	some interphase nuclei are seen which only have a peripheral block of labeling, presumably the late replicating X chromosome which forms the Barr body
Skin Cells XX	one X chromosome in females is late replicating
Fibroblasts XX	sex chromatin body does not replicate or very little, in the first 2.5 hours of the S period, but does replicate as much as euchromatin in middle 3.5 hours and much more than euchromatin in the last 1.6 hours of S period
Fibroblasts XX	
Embryonic fibroblast lymphocytes XX, XY	late replicating X chromosome seen in last few hours of S period, Y chromosome replicates at 3 times the rate of the 21-22 group in the late S period
Fibroblasts XXXXY	three late replicating X chromosomes replicate very little or not at all in first 20 min. of S period, the Y chromosome replication pattern parallels the late X chromosomes
Fibroblasts XX, XY, XXX	in the first half of the S period one X chromosome does not replicate or only very little
Fernandes' amnion strain	
Diploid strains of embryonic source XX	late replicating X chromosome seen in only a few cells in last few hours of S period

AND SEX CHROMOSOME REPLICATION PATTERNS(FIBROBLAST-LIKE CELLS)

Duration of Cell Cycle Periods (Hours)				Method	Reference
G1 Period	S Period	G2 Period	Total		
				labeling with H^3TdR, harvest within ½ to 1 hour	Atkins (1962)
	16	3-5-8 3-4	24+ 17-18	pulse labeling, serial harvest	Atkins and Santesson (1966)
				pulse labeling, immediate harvest observation of sex chromatin labeling in interphase cells	Comings (1967a)
8.5	7.5	3-5-8	18-21	pulse labeling, serial harvest continuous end labeling	Comings (1967b)
				continuous end labeling	Schmid
				continuous end labeling, FUdR synchronization for initiation patterns	Hsu and Lockhart (1964)
				FUdR synchronization for initiation patterns, H^3TdR labeling in the first .16 to 5.0 hours of S period	Priest *et al.* 1967
7.3-10.3	8.5-8.7	2.4	18.2-21.4	autoradiography, microcinematography	Sisken and Kinosita (1961)
(metaphase = 20 min., anaphase = 10 min.)					
4.7	12	2-4-6	18-22	pulse labeling, serial harvest	Moorhead and Defendi (1963)
(from % labeled metaphase curve)					
	7-7.5	3-6	18		
(authors' conclusion)					

HUMAN CELL CYCLE ANALYSIS

Sex Chromosomes Constitution of Lymphoctyes used:	Sex Chromosome Replication
XX	
XX, XY	one X chromosome in females is late replicating and the last chromosome finishing replication
XX, XY	one X chromosome in females is late replicating and the last chromosome finishing replication
XX	one X chromosome in female cells replicates at relatively high rates in the late S period and probably does not replicate in the early S period
XX	a late replicating X chromosome occurs in female cells
XX, XY	one X chromosome of the female and the Y chromosome of the male replicate mostly in the late S period
XX, XY	one X chromosome in female cells is the last chromosome to replicate at high rates, the Y chromosome in the male cells begins early but finishes last
XX	one X chromosome in female cells does not replicate or at very low rates in the early S period
XX, XY	one X chromosome of females replicates latest and at high rates, both X chromosomes replicate throughout the S period, Y chromosome begins late and replicates mostly in the late S period
XX	in the last $\frac{1}{2}$ hour of S period rates of replication are constant but fall off in the last 20 minutes with autosomes preceding the late X chromosome by 5 minutes, late X chromosome replicates at 3 times the rate of autosomes and early X chromosome
XX	both female X chromosomes begin replication before some autosomes
XX, XY	the late replicating X chromosome does not replicate in the early S period, the Y chromosome is not late replicating
XX, XY	
XX, XY	the late replicating X chromosome in females does not replicate in the first half of the S period, the Y chromosome only replicates in the second half of the S period

AND SEX CHROMOSOME REPLICATION PATTERNS (LYMPHOCYTES)

Duration of Cell Cycle Periods (Hours)				Method	Reference
G1 Period	S Period	G2 Period	Total		
		4-5		pulse labeling, serial harvest	Lima-de-Faria *et al.* (1961)
	20-23.5	2.5-6		pulse labeling, serial harvest	German (1962a)
	16-22	3-5-8		pulse labeling, serial harvest	German (1962b)
		2-3		continuous end labeling (3-4 H.) quantitative analysis	Gilbert *et al.* (1962)
				continuous end labeling	Bader *et al.* (1963)
				continuous end labeling	German (1964)
		2-2.5-5	18-22	pulse labeling, serial harvest	Kikuchi and Sandberg (1964)
(% labeled metaphase graph)					
8	13-17		28		
(authors' statement)					
				aminopterin synchronization for initiation patterns	Petersen (1964)
	20	3-5-10	25^+	pulse labeling, serial harvest, grouping of cells with similar labeling patterns from several adjacent harvests	Bianchi and de Bianchi (1965)
				continuous end labeling	Gilbert *et al.* (1965)
				continuous initiation labeling	Mukherjee and Sinha (1965)
4.6	9.6	1.5-3.5-7	17.7	pulse labeling, serial harvest (many cells used)	Cave (1966)
		2-3.5-6		pulse labeling, serial harvest	Cave (1967)
	$7\text{-}15\text{-}15^+$	2-3-5	24	pulse labeling, serial harvest	Sofuni and Sandberg (1967)

TABLE

PATTERN OF REPLICATION OF ABNORMAL

Sex Chromosome Constitution of Lymphocytes used:	Sex Chromosome Replication
XO, XO/XX/XXX	all X chromosomes, except one per cell, are late replicating, one X chromosome replicates throughout the S period with most autosomes
XXXXY	3 out of 4 X chromosomes are late replicating
XXX/XXXXY	all but one X chromosome per cell is late replicating, the other is early replicating
XYY, XXYY, XY	one late X chromosome in XXYY; all Y chromosomes replicate at 4 times the rate of the chromosomes in 21-22 group in the late S period
XXX, XXXY, XXXXX, X/iso-X, XO/iso-X	all but one X chromosome are late replicating, the iso-X chromosome is always late replicating, Barr body labeling, Y chromosome finishes replication before the late X chromosome
XO/X iso-X	the iso-X crhomosome is always late replicating
X/iso-X (long arm)	the abnormal iso-X chromosome is always late replicating
XXX XO/X iso-X (long arm)	2 out of 3 X chromosomes are late replicating, iso-X chromosome is late replicating
X/iso-X	X chromosomes of the female begin replication before some autosomes
X/iso-X (and others)	iso-X chromosome always heavily labeled in the late S period and lightly labeled or unlabeled in the early S period
$XO/XX_R/XX_RX_R$ (X_R means ring X chromosome)	ring X chromosome is always a late replicating sex chromosome

SEX CHROMOSOME CONSTITUTIONS IN HUMAN LYMPHOCYTES

	Method	Reference
	pulse labeling, serial harvest	Morishima *et al.* (1962)
	continuous end labeling, quantitative analysis	Rowley *et al.* (1963)
	continuous end labeling	Mukherjee *et al.* (1964)
	continuous end labeling	Boczkowski and Casey (1967)
	colchicine harvest at metaphase of cells labeled in the late S period	Grumbach *et al.* (1963)
	continuous end labeling	Miller *et al.* (1963)
	continuous end labeling quantitative analysis	Muldal *et al.* (1963)
	continuous end labeling	Giannelli (1963)
	continuous initiation labeling	Mukherjee *et al.* (1966)
	FUdR synchronization, pulse labeling with H^3TdR at beginning and end of the S period	Ockey *et al.* (1966)
	continuous end labeling	Rowley *et al.* (1964)

IX FIGURES

FIGURE 1.

The cell cycle consists of the period from one mitosis to the next mitosis with the interphase subdivided into G1, S and G2 periods. The S period is the interval when DNA synthesis occurs. Daughter cells may also pass through these periods of the cell cycle.

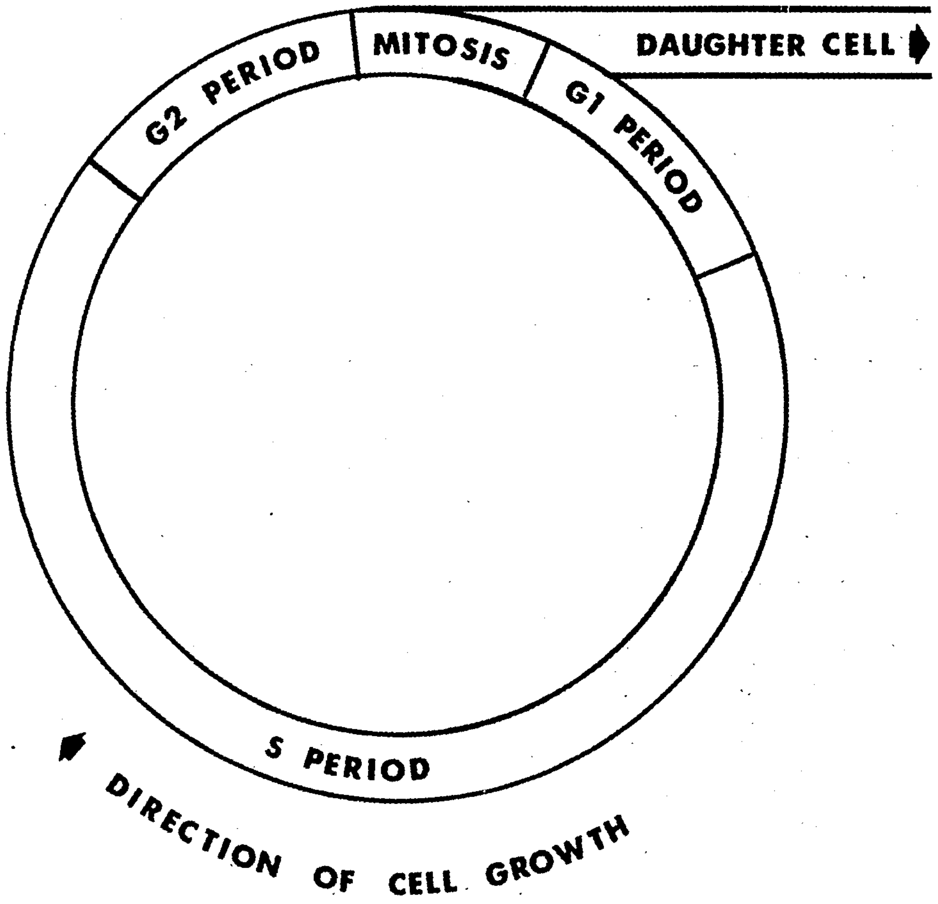

MITOSIS
DAUGHTER CELL
G1 PERIOD
S PERIOD
G2 PERIOD
DIRECTION OF CELL GROWTH

FIGURE 1

FIGURE 2.

The percentage of labeled metaphase figures expected in serial harvests after pulse labeling the culture with H^3TdR. The duration of the G2 period is measured from time zero to the time of 50 per cent labeling on the ascending slope of the first wave. The duration of the S period is measured from the 50 per cent labeling point on the ascending slope to the 50 per cent point on the descending slope. The time required for a complete cell cycle is obtained by measuring from the mid point of the first wave to the mid point of the second wave. The duration of the G1 period is obtained by subtracting the duration of the G2 plus S periods from the duration of the total cell cycle. The time intervals of the G1 and G2 periods both include some of the time taken for mitosis.

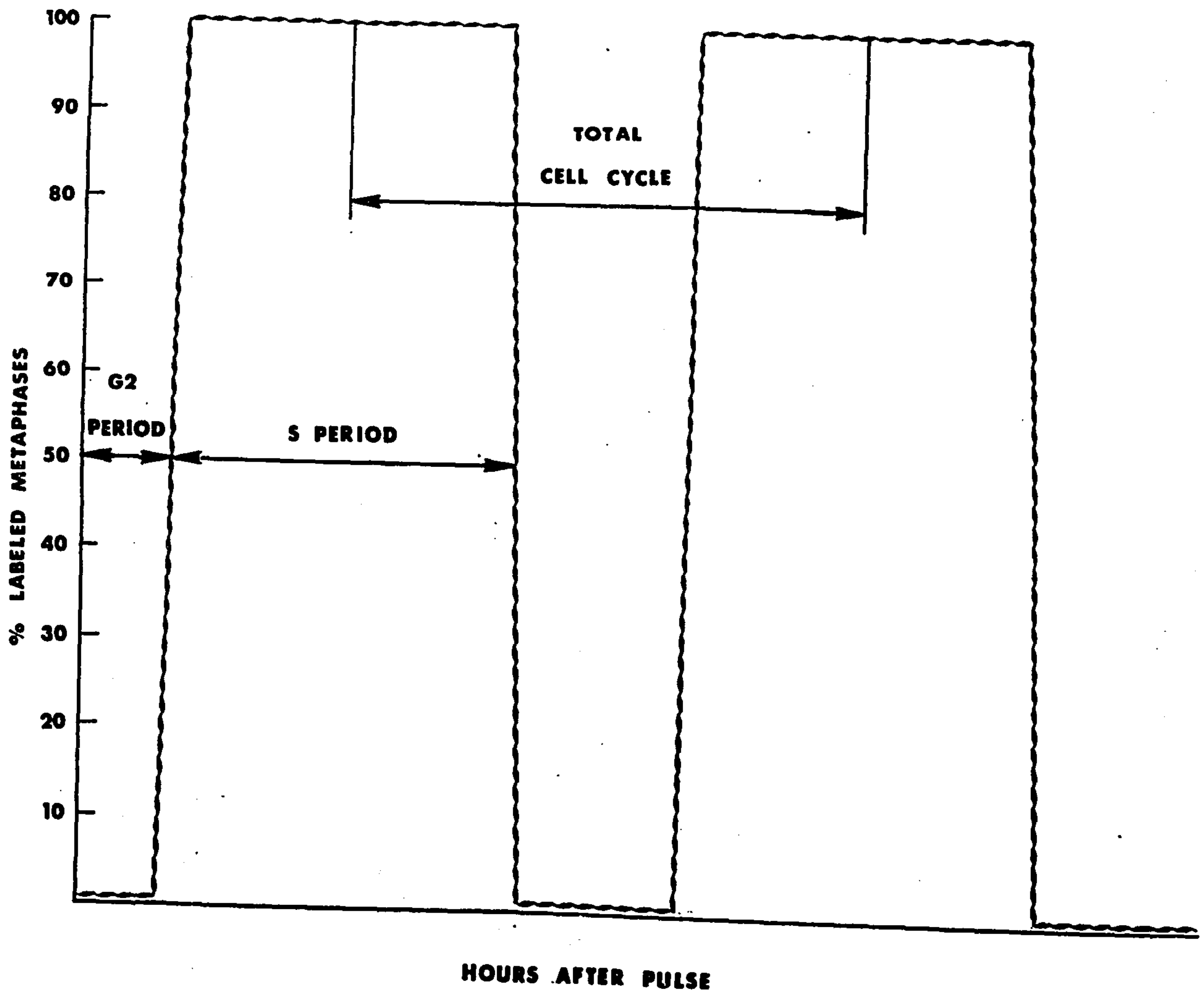

% LABELED METAPHASES
100
90
80
70
60
50
40
30
20
10
G2
PERIOD
S PERIOD
TOTAL
CELL CYCLE
HOURS AFTER PULSE

FIGURE 2

FIGURE 3.

The percentage of labeled metaphase figures observed in serial harvests after pulse labeling the culture of bovine female lymphocytes with H^3TdR. The G2 and S periods have average durations of 2 and 8 hours, respectively. The total cell cycle required about 12 hours. The average duration of the G1 period was about 2 hours. On this graph the S period began at 10 hours after pulse and ended at 2 hours after pulse. Each point on the graph is based on the observation of the autoradiographic labeling of 200 metaphase figures. The data were listed in Table III.

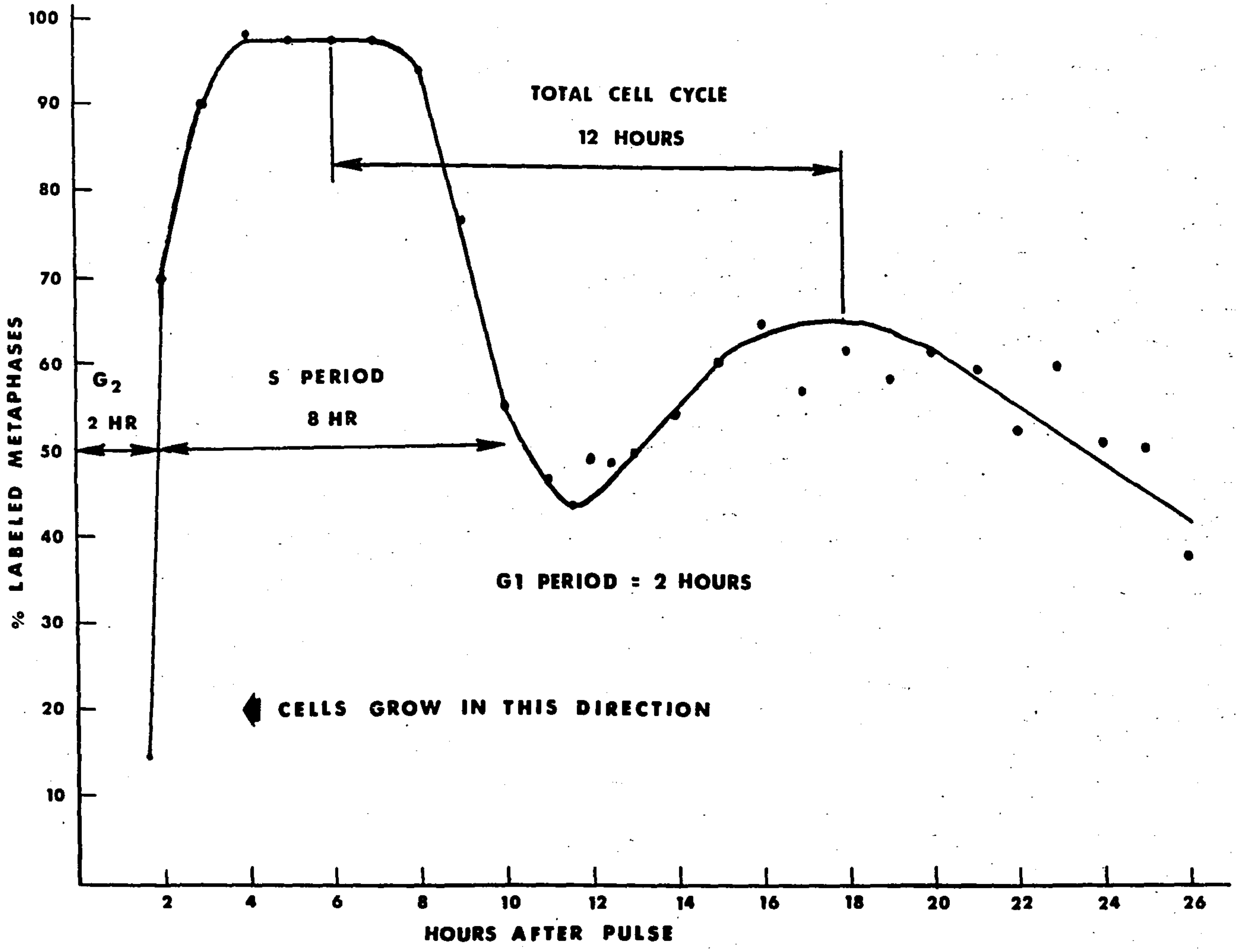

100
90
80
70
60
50
40
30
20
10
% LABELED METAPHASES
2
4
6
8
10
12
14
16
18
20
22
24
26
HOURS AFTER PULSE
TOTAL CELL CYCLE
12 HOURS
G_2
2 HR
S PERIOD
8 HR
G1 PERIOD = 2 HOURS
CELLS GROW IN THIS DIRECTION

FIGURE 3

FIGURE 4.

The average grain densities on the X chromosomes (•————•) and on the Y chromosomes (•∼∼∼∼•) in metaphase figures of male bovine lymphocytes in each harvest after a pulse with H^3TdR. The graph is read from the right to the left with the S period beginning at 10 hours and ending at 2 hours. The data for these graphs were listed in Table I.

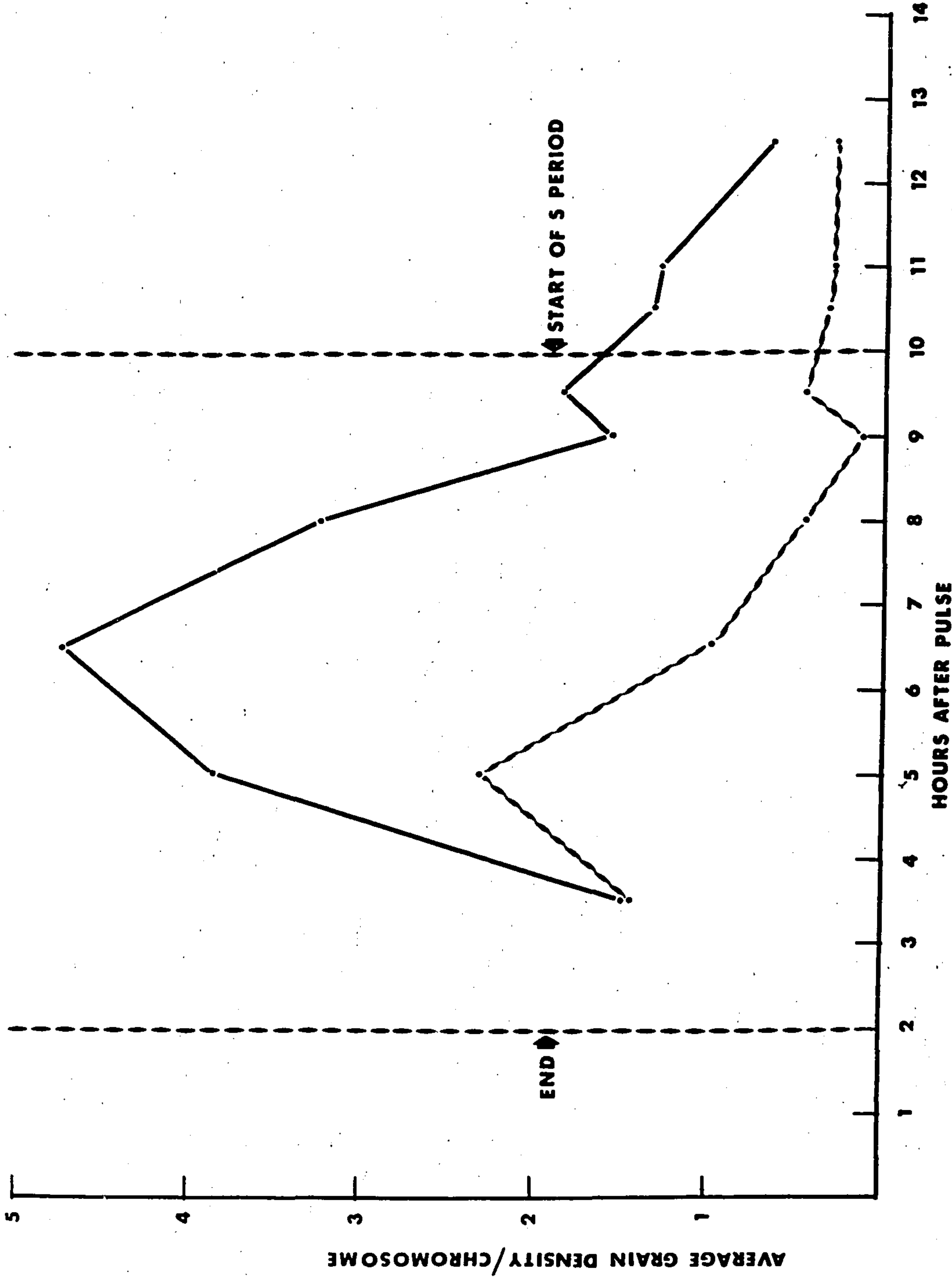

START OF S PERIOD
END
1
2
3
4
5
6
7
8
9
10
11
12
13
14
HOURS AFTER PULSE
1
2
3
4
5
AVERAGE GRAIN DENSITY/CHROMOSOME

FIGURE 4

FIGURE 5.

The relative cumulative amounts of DNA synthesized by the X chromosome (•————————•) and by the Y chromosome (•--------•) as the S period progressed. These graphs were obtained by integrating the graphs in Figure 4 from the beginning of the S period to each point in the S period. Since the axes in Figure 4 were expressed in grains and hours, the integrals of the graphs in Figure 5 could be expressed in grain-hours.

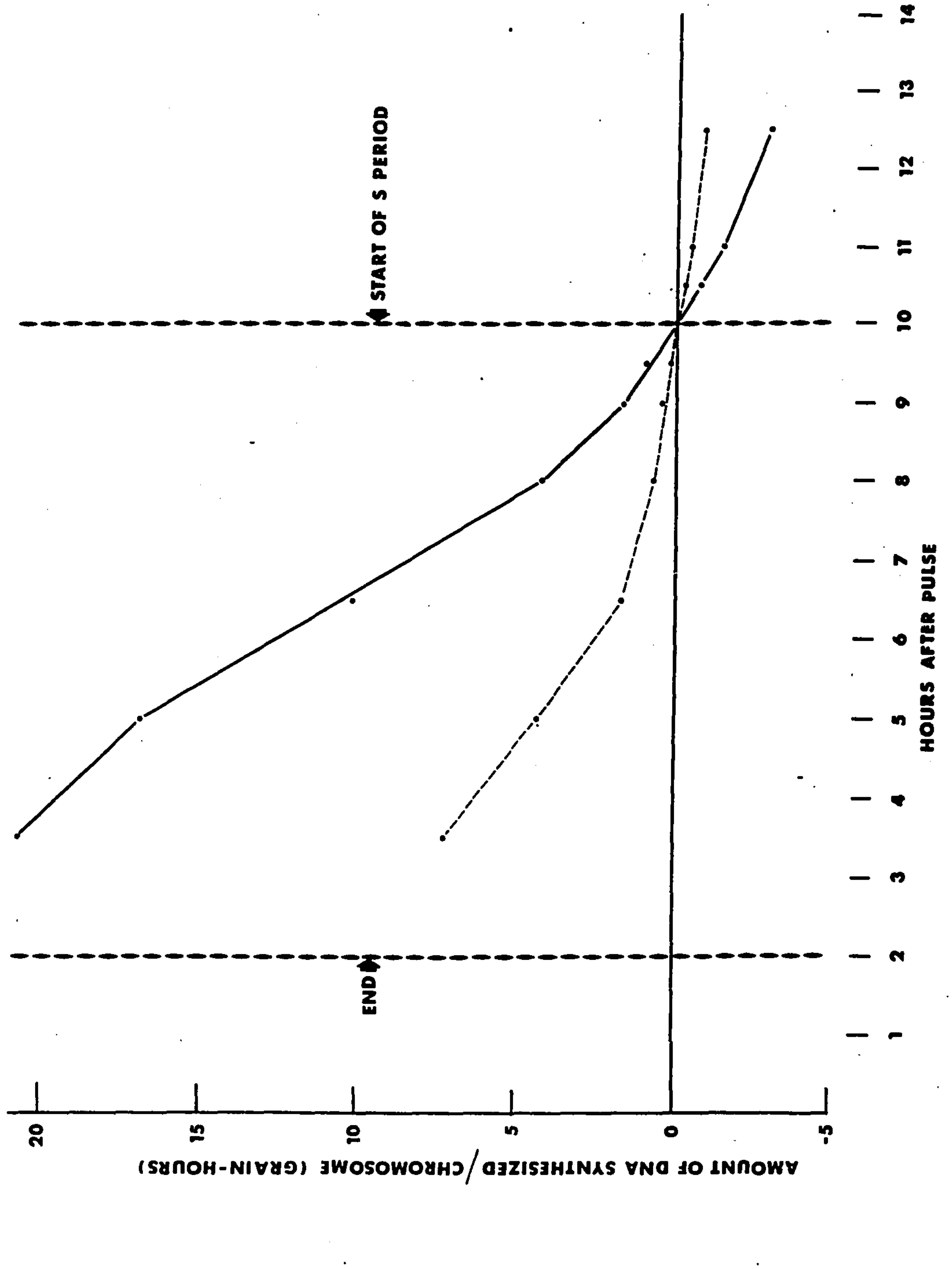

START OF S PERIOD
END
HOURS AFTER PULSE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
AMOUNT OF DNA SYNTHESIZED/CHROMOSOME (GRAIN-HOURS)
20
15
10
5
0
-5

FIGURE 5

FIGURE 6.

The uncorrected average grain densities per chromosome class in each harvest after pulse labeling the bovine female lymphocyte culture with H^3TdR.

The 'heavily' labeled X chromosome class (•———•),

the 'lightly' labeled X chromosome class (• – – – – •),

the 'heavily' labeled longest autosome class (•〰〰〰•),

and the 'lightly' labeled longest autosome class (•〰 〰 〰•).

The data used to plot these curves were listed in Table II.

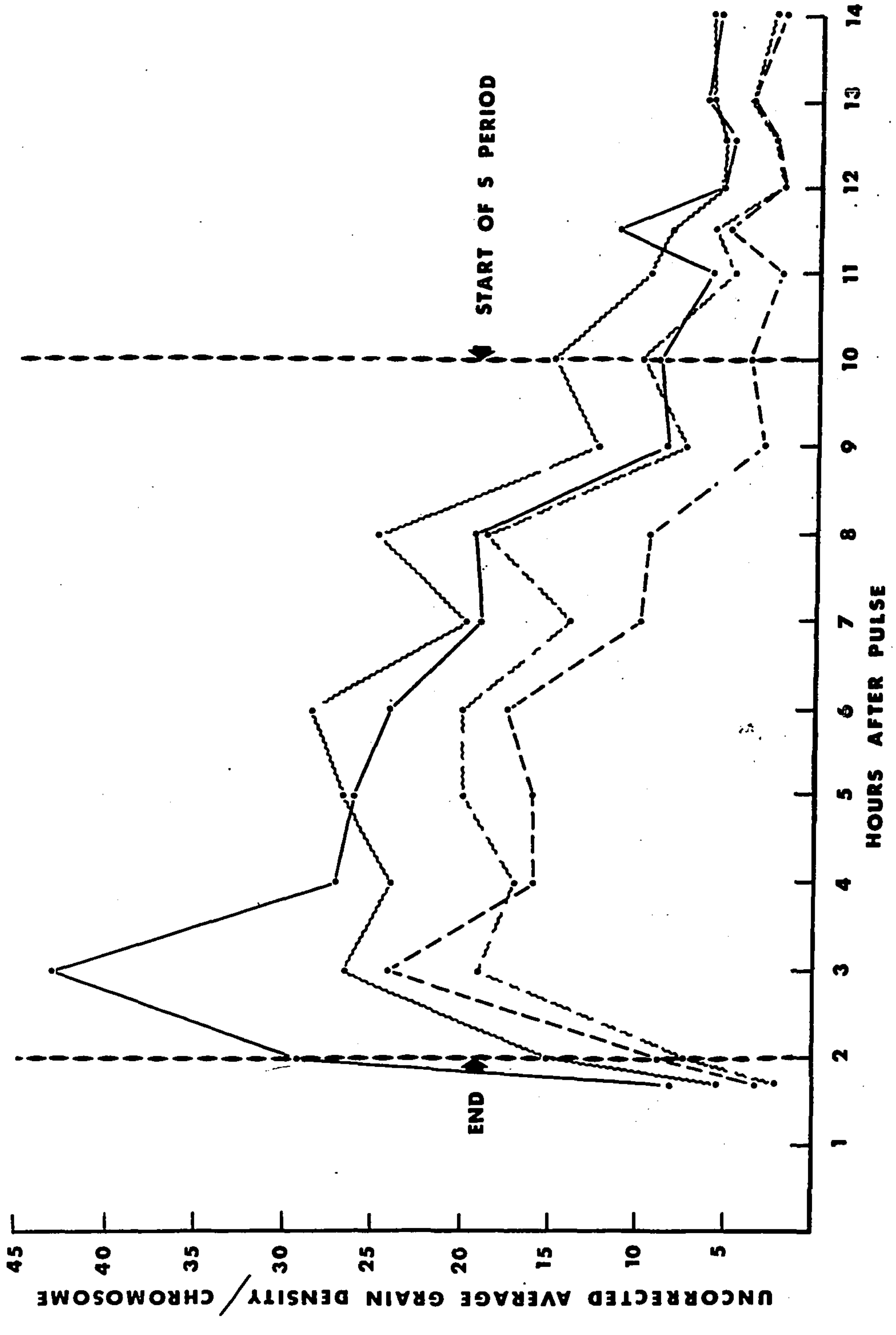

START OF S PERIOD
END
UNCORRECTED AVERAGE GRAIN DENSITY / CHROMOSOME
45
40
35
30
25
20
15
10
5
HOURS AFTER PULSE
1
2
3
4
5
6
7
8
9
10
11
12
13
14

FIGURE 6

FIGURE 7.

The percentage of X chromosome pairs in the L, S and D categories in each harvest after pulse labeling the bovine female lymphocyte culture with H^3TdR. The L category (•— — — — —•) consisted of pairs of X chromosomes that had a total of 9 or fewer grains overlying them. The S category (•————•) consisted of pairs that had more than 9 grains on them and that had grain densities on each of the two X chromosomes that were not significantly different as determined with a chi-square test. The D category (•- - - - - - - -•) consisted of pairs that had more than 9 grains on them and that had grain densities on the two chromosomes of the pairs that were significantly different. The data for these graphs were given in Table II.

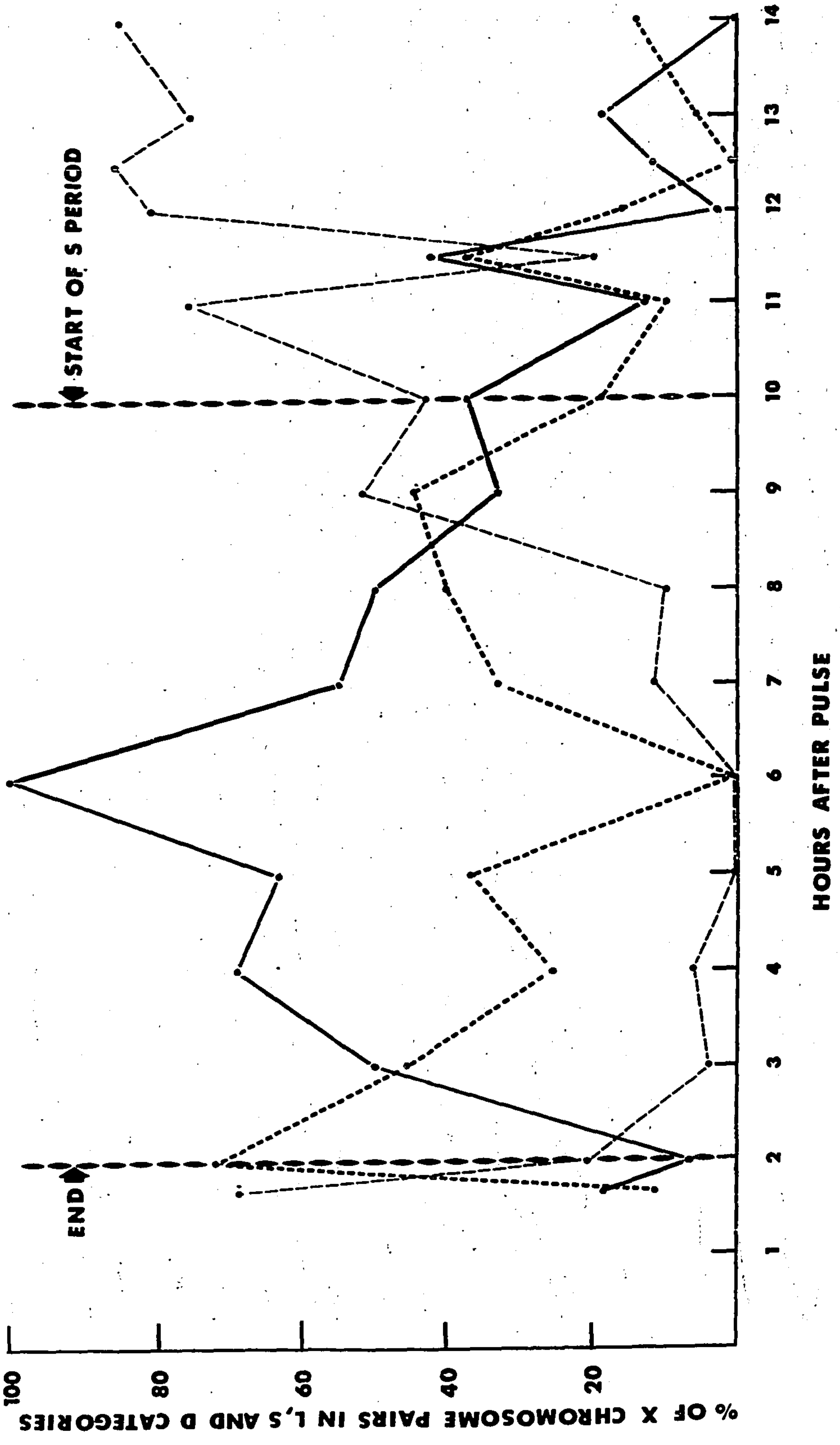

START OF S PERIOD
END
HOURS AFTER PULSE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
% OF X CHROMOSOME PAIRS IN L,S AND D CATEGORIES
100
80
60
40
20

FIGURE 7

FIGURE 8.

The percentage of the longest autosome pairs in the L, S and D categories in each harvest after pulse labeling the bovine female lymphocyte culture with H^3TdR. The L category (•– – – – – – –•), the S category (•————————•) and the D category (•---------•), were defined in the same way as in the case of the X chromosome pairs. The data for these graphs were included in Table II.

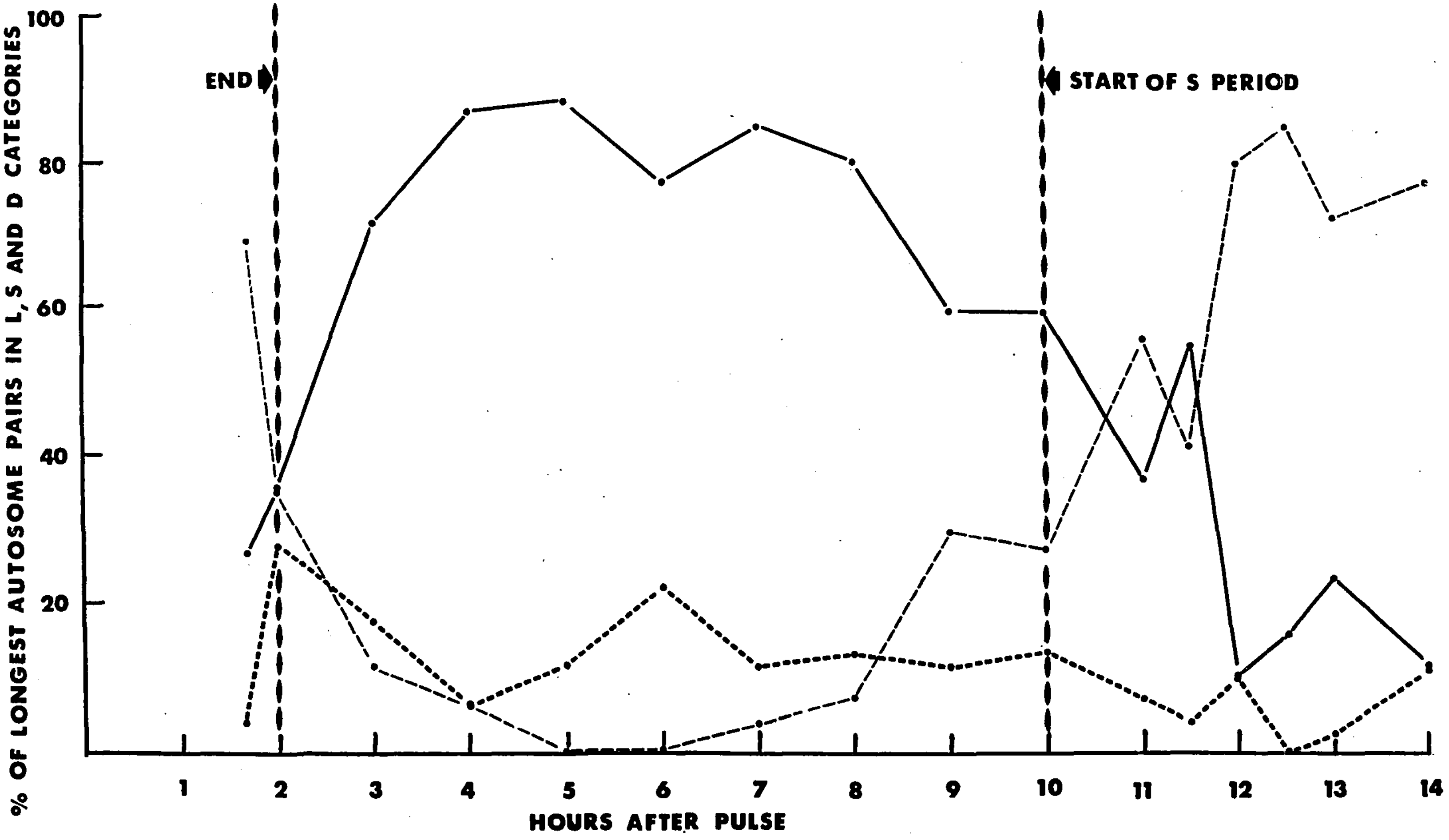

END
START OF S PERIOD
% OF LONGEST AUTOSOME PAIRS IN L, S AND D CATEGORIES
100
80
60
40
20
1
2
3
4
5
6
7
8
9
10
11
12
13
14
HOURS AFTER PULSE

FIGURE 8

FIGURE 9.

The corrected average grain densities per chromosome class in each harvest after pulse labeling the bovine female lymphocyte culture with H^3TdR.

The 'heavily' labeled X chromosome class (•————•),
the 'lightly' labeled X chromosome class (•- - - - - -•),
the 'heavily' labeled longest autosome class (•∼∼∼∼∼∼•),
and the 'lightly' labeled longest autosome class (•∼∼ ∼∼ ∼∼•).
Note that at 6 hours after pulse the average grain densities of the 'heavily' and 'lightly' labeled X chromosome classes were equal but were quite different in other harvests. This 6 hour point was referred to as the 'switch-over' point. The grains on the longest autosomal classes, however, were not equal at 6 hours but were not very different throughout the S period. There did not appear to be anything like a 'switch-over' point in the case of the autosomes.

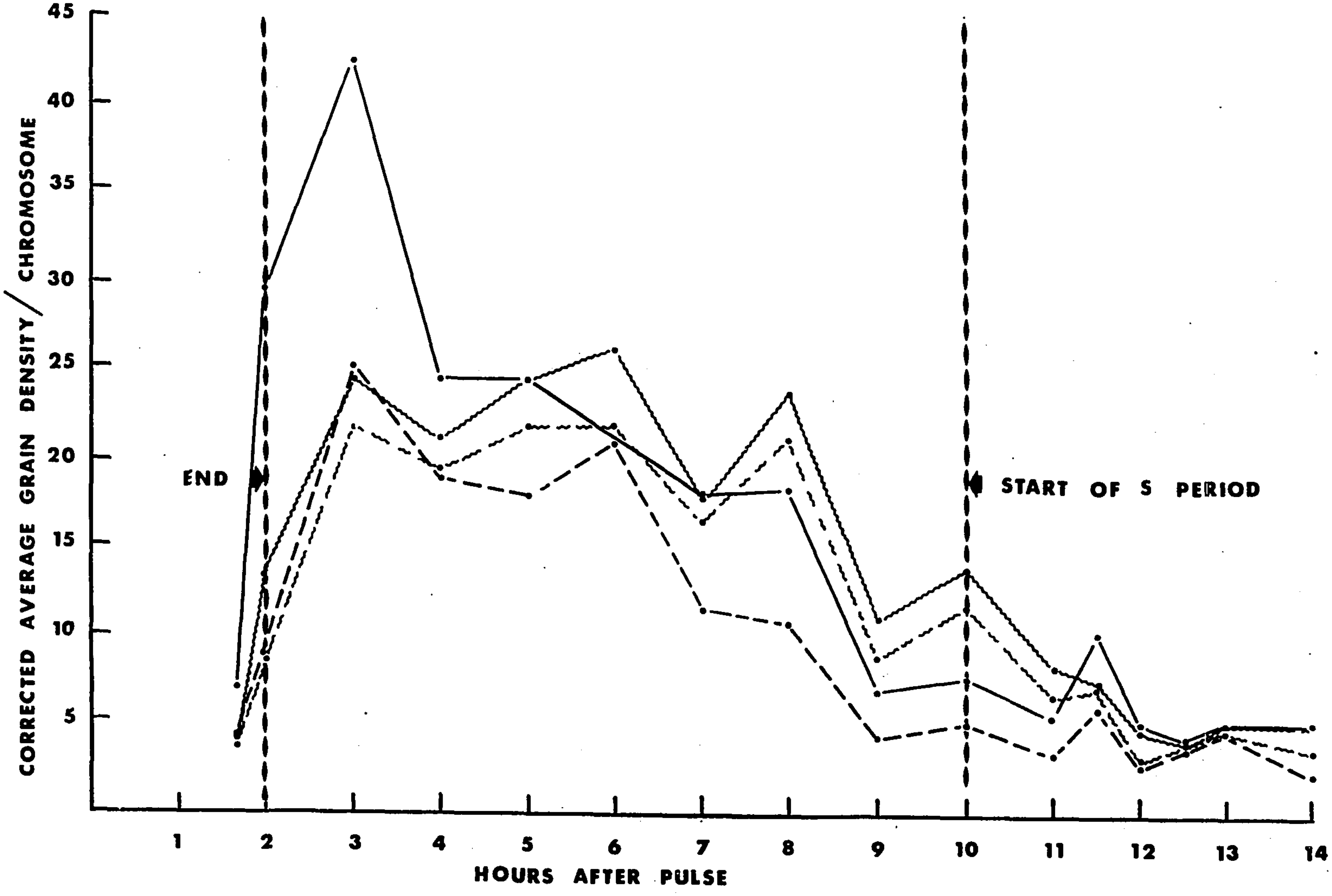

45
40
35
30
25
20
15
10
5
CORRECTED AVERAGE GRAIN DENSITY / CHROMOSOME
END
START OF S PERIOD
1
2
3
4
5
6
7
8
9
10
11
12
13
14
HOURS AFTER PULSE

FIGURE 9

FIGURE 10.

The relative cumulative amounts of DNA synthesized by each of the two X chromosomes as the S period progressed in female bovine lymphocytes. These graphs were obtained by integrating the X chromosome graphs in Figures 6 and 9 from the beginning of the S period to each point in the S period. Cumulative integral graphs of the 'early' replicating X chromosome using uncorrected average grain densities (◆– – – – – – –◆) and using corrected average grain densities (◆————◆). Cumulative integral graphs of the 'late' replicating X chromosome using uncorrected average grain densities (●– – – – – – –●) and using corrected average grain densities (●————●).

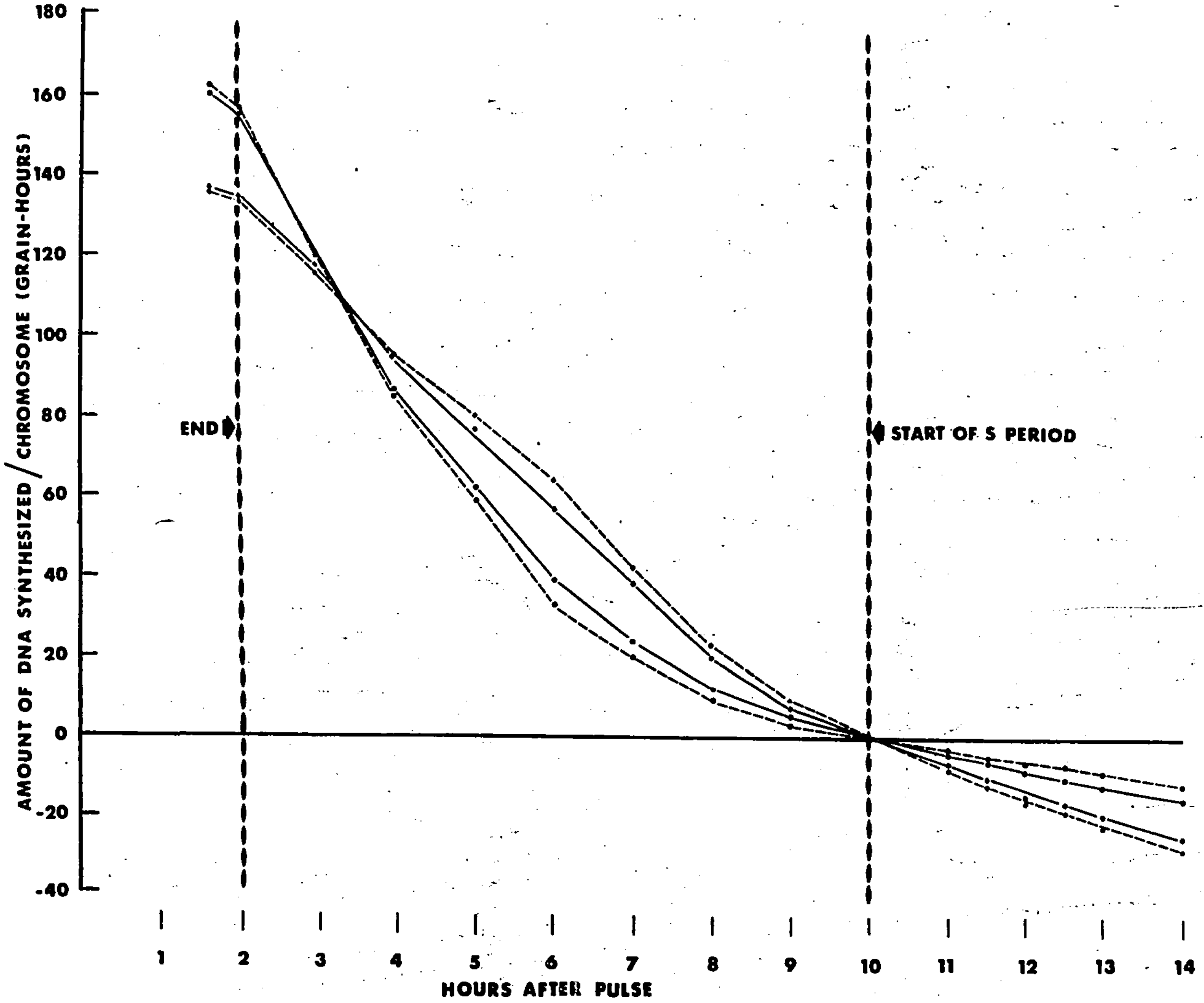

AMOUNT OF DNA SYNTHESIZED / CHROMOSOME (GRAIN-HOURS)
180
160
140
120
100
80
60
40
20
0
-20
-40
END
START OF S PERIOD
1
2
3
4
5
6
7
8
9
10
11
12
13
14
HOURS AFTER PULSE

FIGURE 10

FIGURE 11.

The relative cumulative amounts of DNA synthesized by each of the two longest autosomes as the S period progressed in female bovine lymphocytes. These graphs were obtained by integrating the two longest autosome graphs in Figures 6 and 9 from the beginning of the S period to each point in the S period. Cumulative integral graphs of the 'heavily' labeled longest autosome using uncorrected average grain densities (◆∿∿∿∿∿◆) and corrected average grain densities (◆———◆). Cumulative integral graphs of the 'lightly' labeled longest autosome using uncorrected average grain densities (●∿∿∿∿∿●) and corrected average grain densities (●———●).

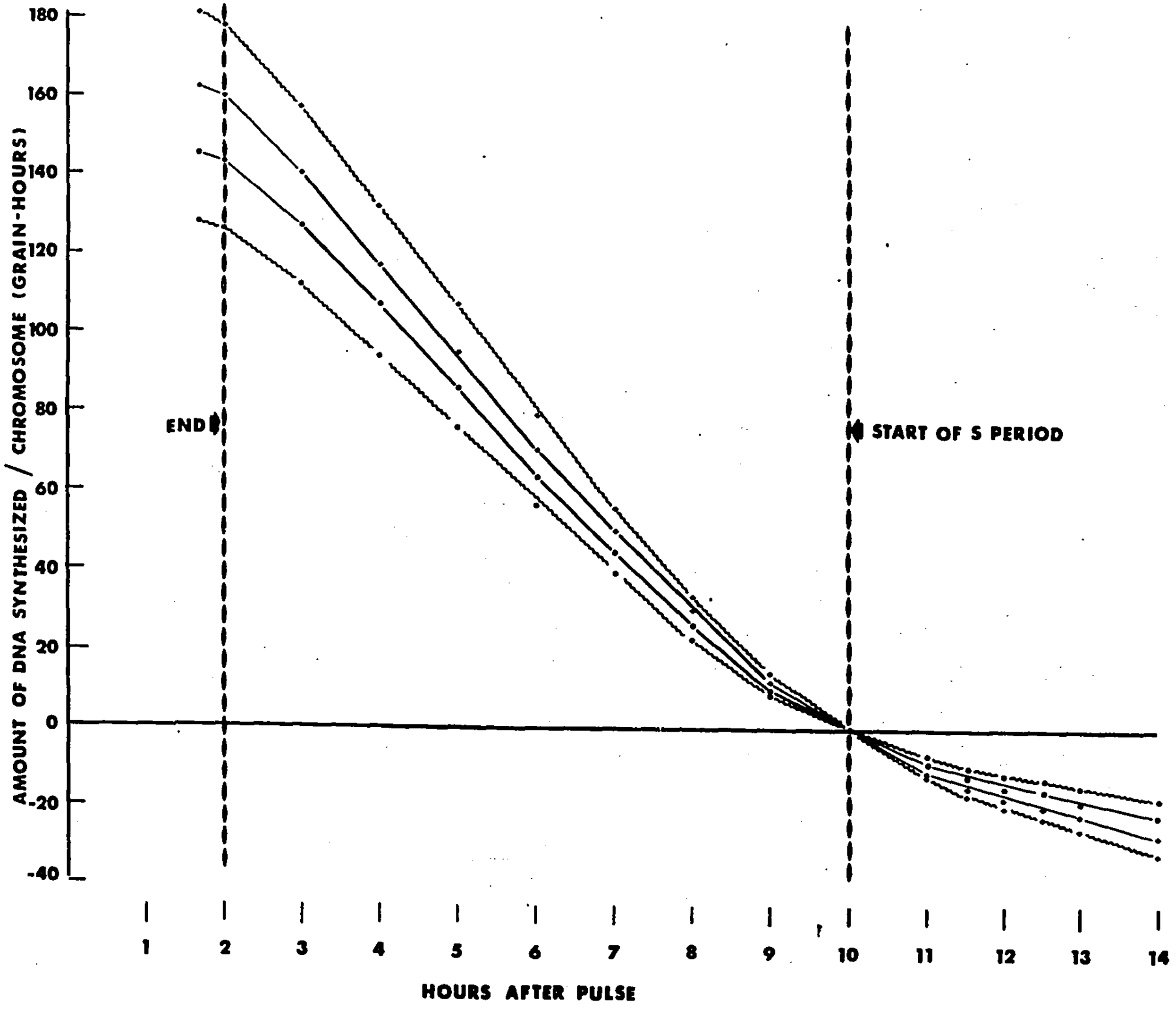

180
160
140
120
100
80
60
40
20
0
-20
-40
AMOUNT OF DNA SYNTHESIZED / CHROMOSOME (GRAIN-HOURS)
END
START OF S PERIOD
1
2
3
4
5
6
7
8
9
10
11
12
13
14
HOURS AFTER PULSE

FIGURE 11

FIGURE 12.

The percentage of replication completed by each chromosome as the S period progressed. The heterochromatic or 'late' replicating X chromosome using uncorrected average grain densities (•- - - - - - - - -•) and using corrected average grain densities (•————•). The euchromatic or 'early' replicating X chromosome using uncorrected average grain densities (◆- - - - - - - - -◆) and corrected average grain densities (◆————◆). The 'heavily' labeled longest autosome using uncorrected and corrected average grain densities (o〰〰〰o). The 'lightly' labeled longest autosome using uncorrected and corrected average grain densities (o∼∼∼∼o). Both of the longest autosomes are composed of euchromatin. These graphs were obtained by converting the cumulative integral of each chromosome graph at each point to a percentage of the integral obtained at 2 hours on the same graph in Figures 10 and 11.

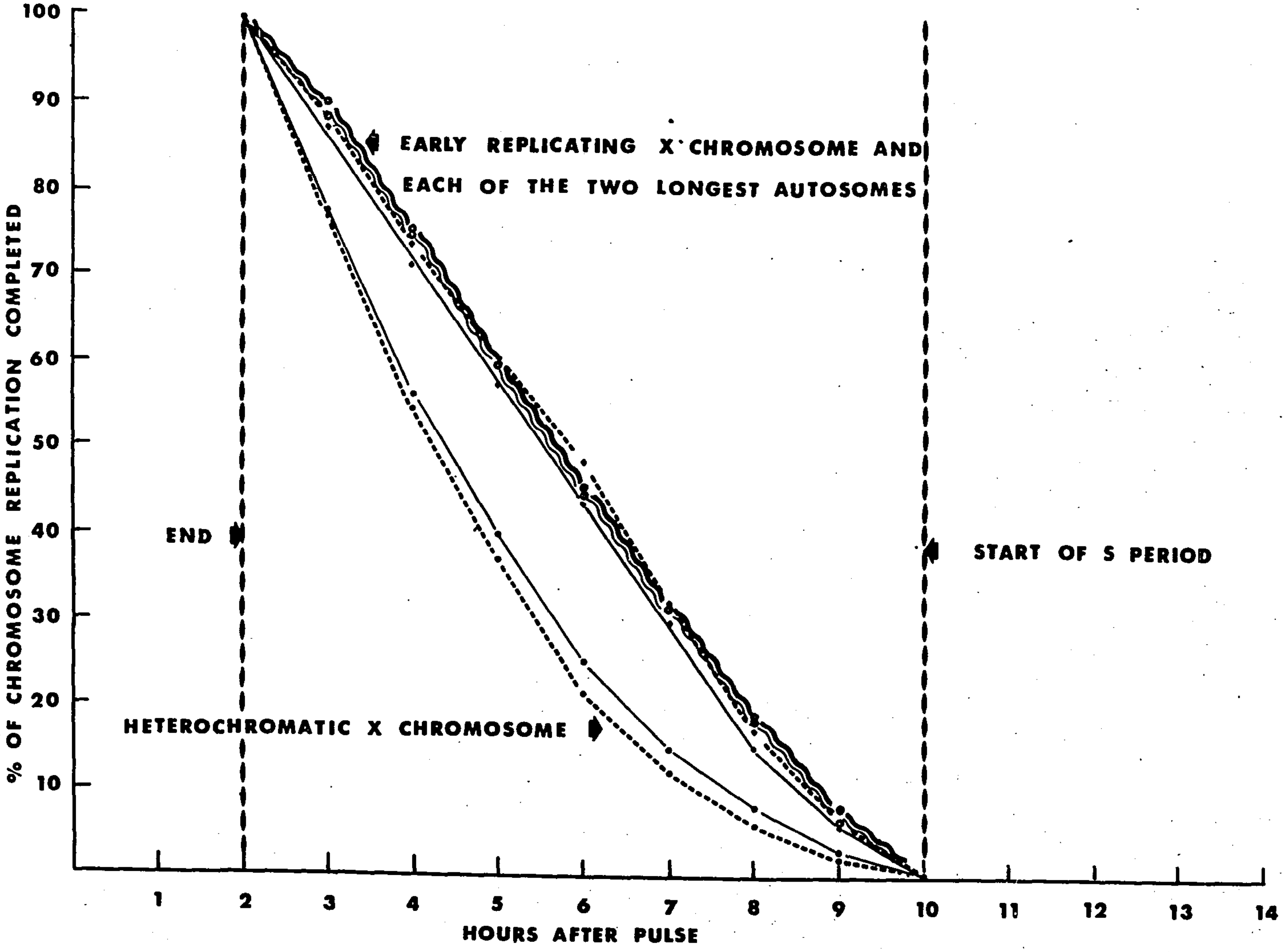
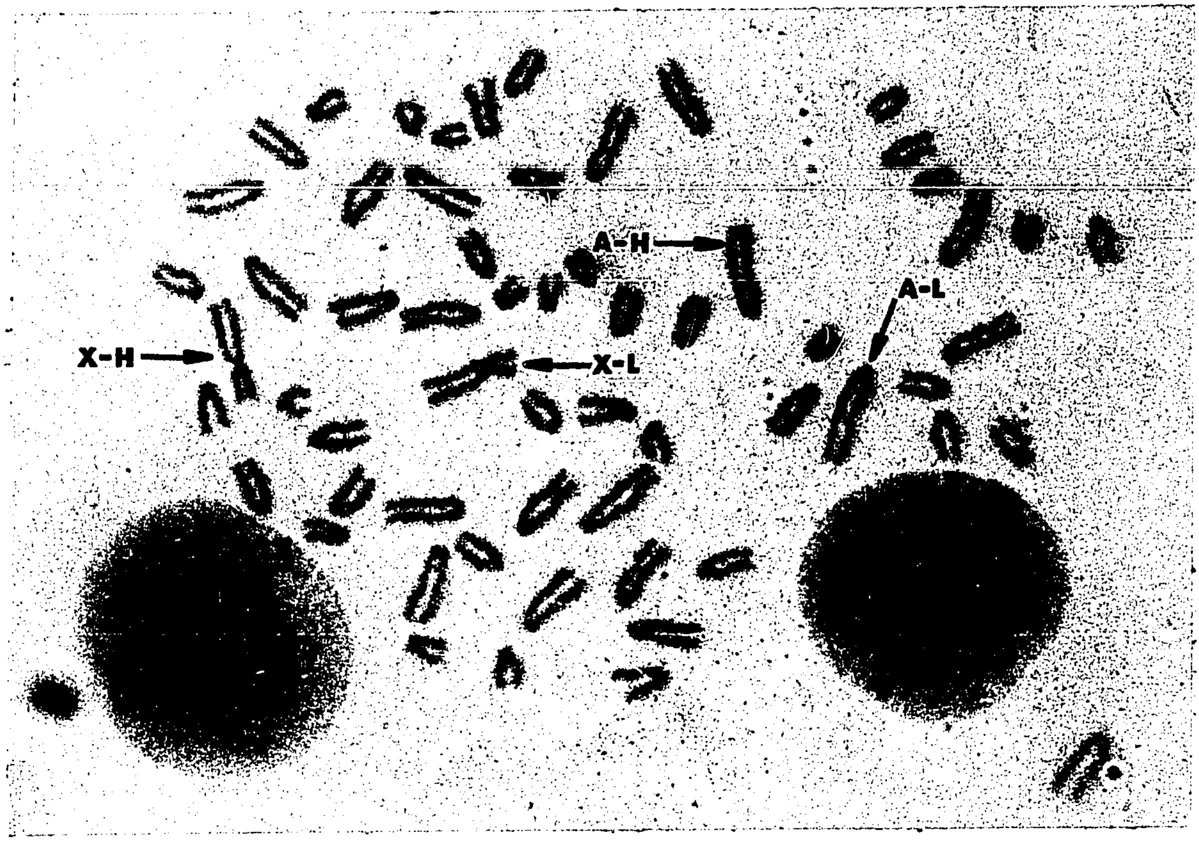

EARLY REPLICATING X CHROMOSOME AND EACH OF THE TWO LONGEST AUTOSOMES
END
START OF S PERIOD
HETEROCHROMATIC X CHROMOSOME
% OF CHROMOSOME REPLICATION COMPLETED
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
HOURS AFTER PULSE

FIGURE 12

X PLATES

PLATE 1

Photograph and autoradiograph of a typical metaphase cell of a bovine female pulse labeled with H^3TdR during the late S period (Table II, 3 hours after pulse, cell no. 3). Note that the lightly labeled X chromosome (X-L), the heavily labeled longest autosome (A-H) and the lightly labeled longest autosome (A-L) had about the same grain density (29, 24 and 20 grains, respectively). The heavily labeled X chromosome (X-H) with approximately 80 grains, was significantly more heavily labeled than the other three chromosomes. This pattern of labeling with a relatively heavily labeled X chromosome is the well known late replication pattern.


A-H
A-L
X-H
X-L

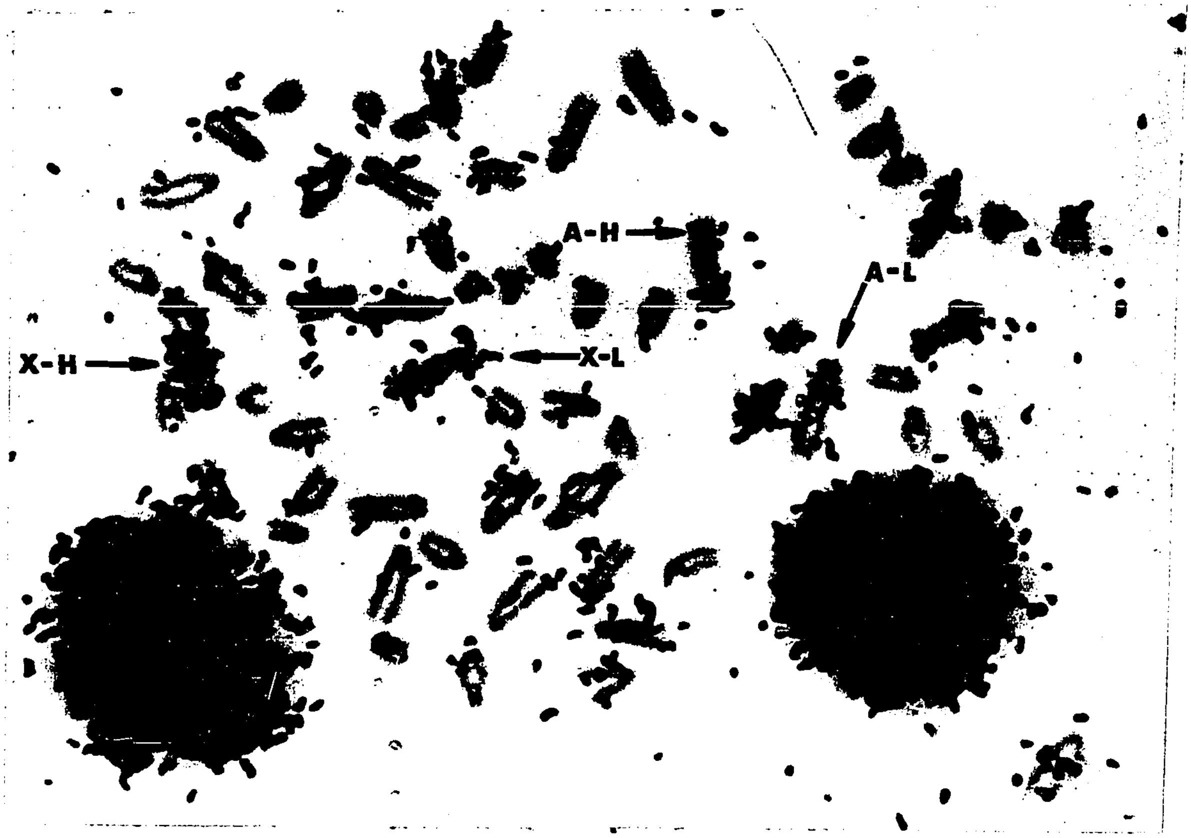
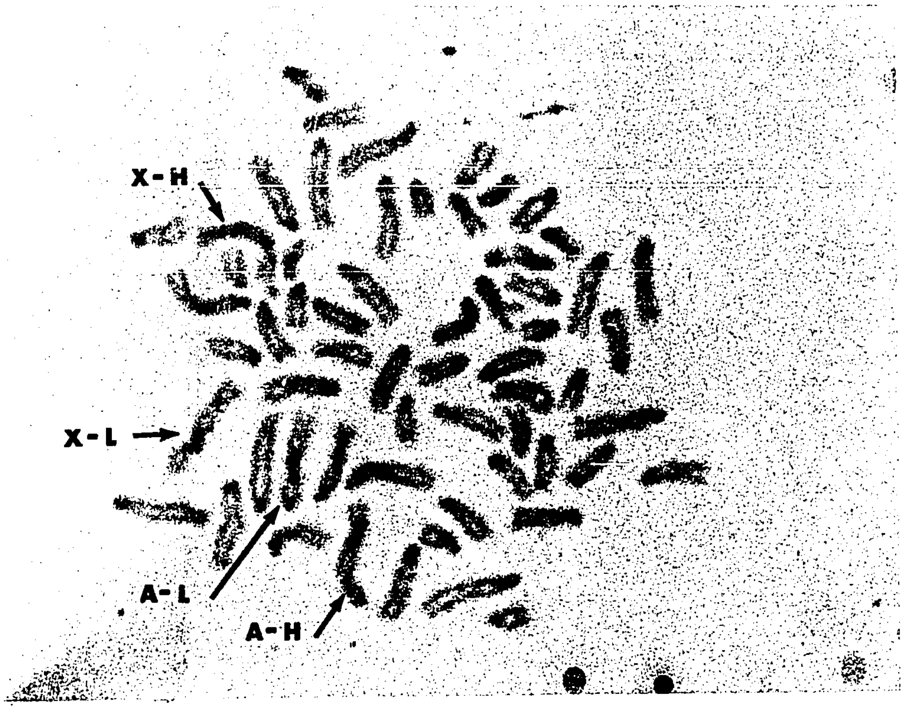

A-H
A-L
X-H
X-L

PLATE 2

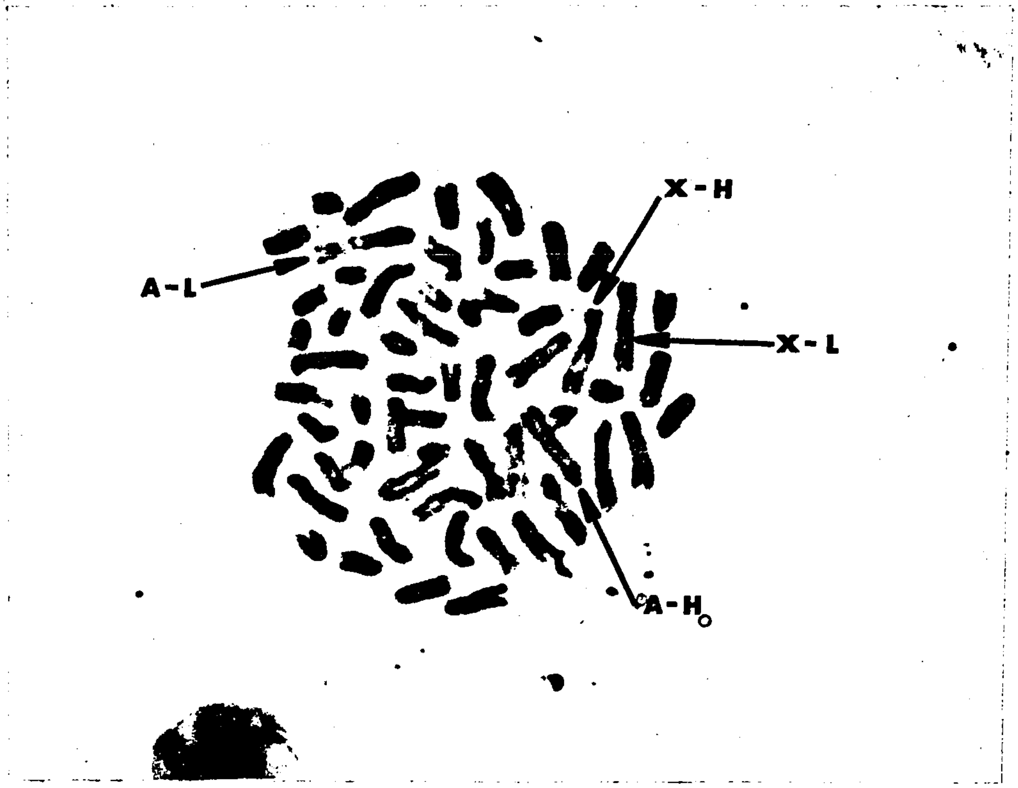
Photograph and autoradiograph of a metaphase cell of a bovine female pulse labeled with H^3TdR during the <u>early</u> S period (Table II, 8 hours after pulse, cell no. 1). Note that the heavily labeled X chromosome (X-H), the lightly labeled X chromosome (X-L), the heavily labeled longest autosome (A-H) and the lightly labeled longest autosome (A-L) had about the same grain density (26, 27 and 26 grains, respectively). The lightly labeled X chromosome (X-L) with approximately 2 grains was significantly less labeled than the other three chromosomes. This type of labeling pattern was found in a large proportion of metaphase cells labeled in the early S period.


X-H
X-L
A-L
A-H


X-H
X-L
A-L
A-H

PLATE 3

Photograph and autoradiograph of a typical metaphase cell of a bovine female most frequently observed among metaphase cells labeled during the mid S period but also at lower frequencies in many other parts of the S period. (Table II, 6 hours after pulse, cell no. 6). The heavily labeled X chromosome (X-H), the lightly labeled X chromosome (X-L), the heavily labeled longest autosome (A-H) and the lightly labeled longest autosome (A-L) had about the same labeling intensity (24, 14, 25 and 18 grains, respectively).


X-H
A-L
X-L
A-H₀

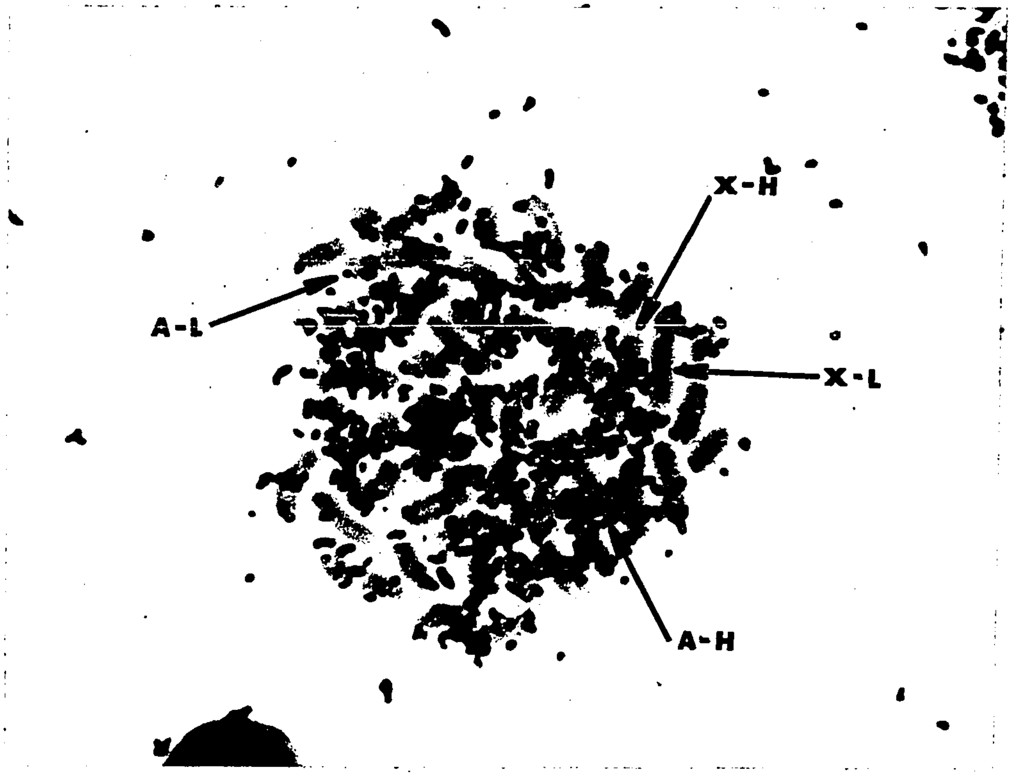
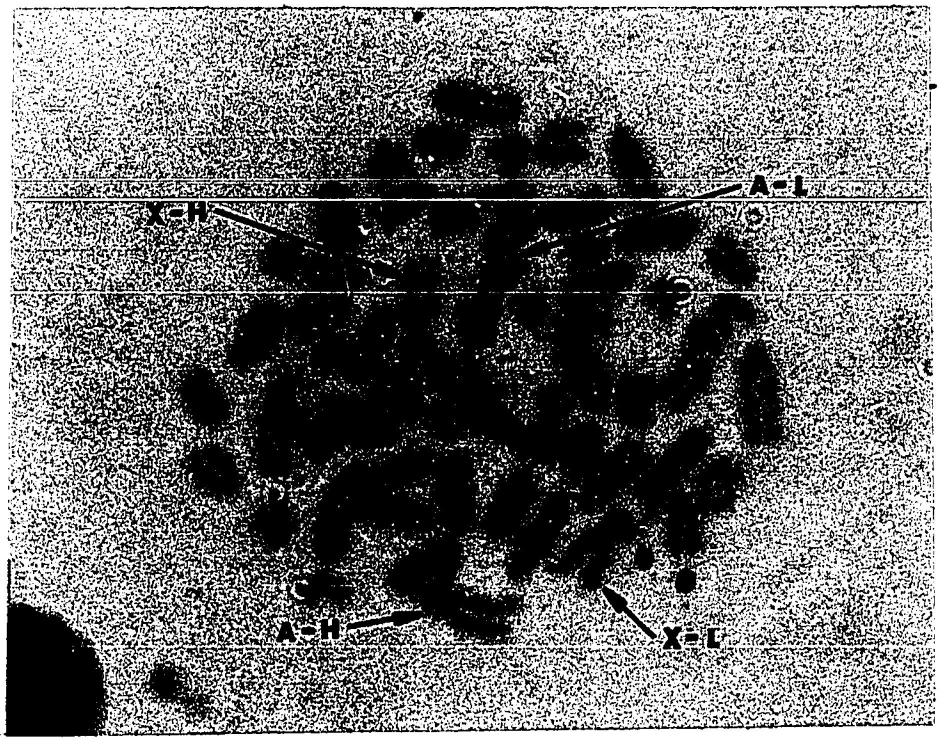

X-H
A-L
X-L
A-H

PLATE 4

Photograph and autoradiograph of a lightly labeled bovine female metaphase cell pulse labeled with H^3TdR (Table II, 12 hours after pulse, cell no. 26). The heavily labeled X chromosome (X-H), the lightly labeled X chromosome (X-L), the heavily labeled longest autosome (A-H) and the lightly labeled longest autosome (A-L) had 5, 2, 2 and 2 grains respectively. This type of light labeling was observed among metaphase cells that were not in the S period during the pulse.


X-H
A-L
A-H
X-L

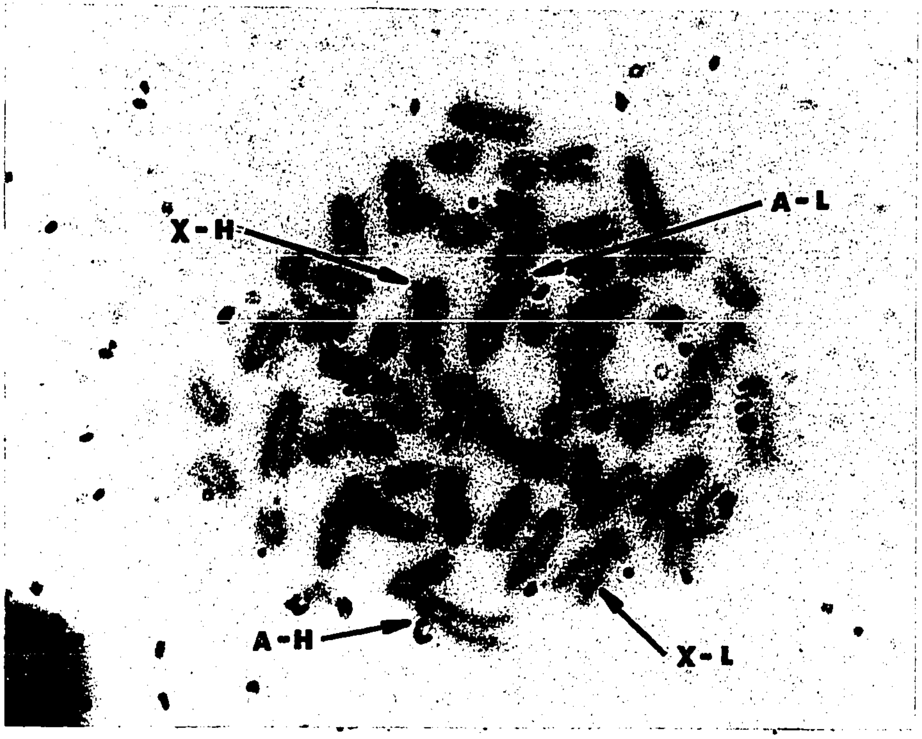

X-H
A-L
A-H
X-L

PLATE 5

Photograph and autoradiograph of a bovine female metaphase cell harvested at the second metaphase after pulse labeling with H^3TdR (Table II, 14 hours after pulse, cell no. 3). Notice chromosomes with single-chromatid labeling (S). The heavily labeled X chromosome (X-H), the lightly labeled X chromosome (X-L), the heavily labeled longest autosome (A-H) and the lightly labeled longest autosome (A-L) had 21, 6, 33 and 18 grains, respectively.

PLATE 5

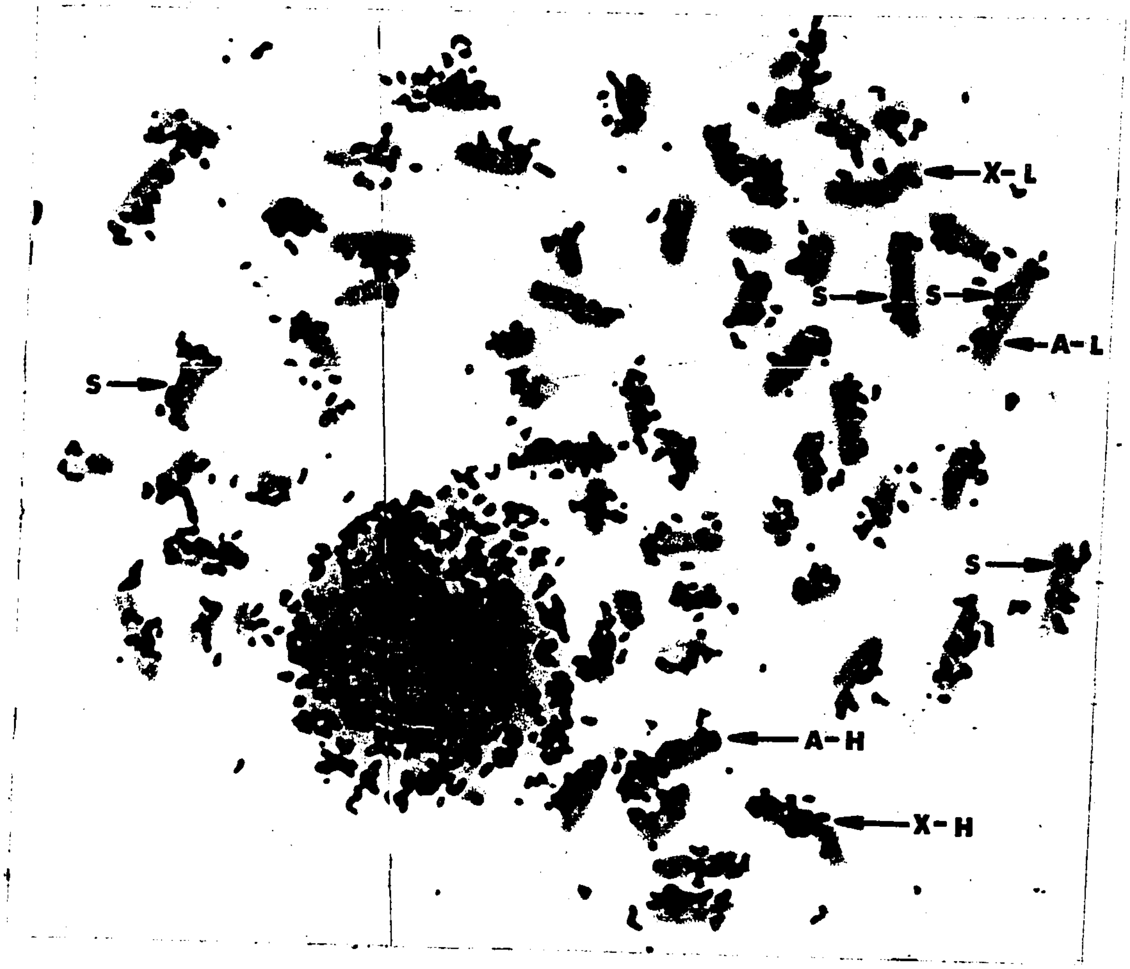
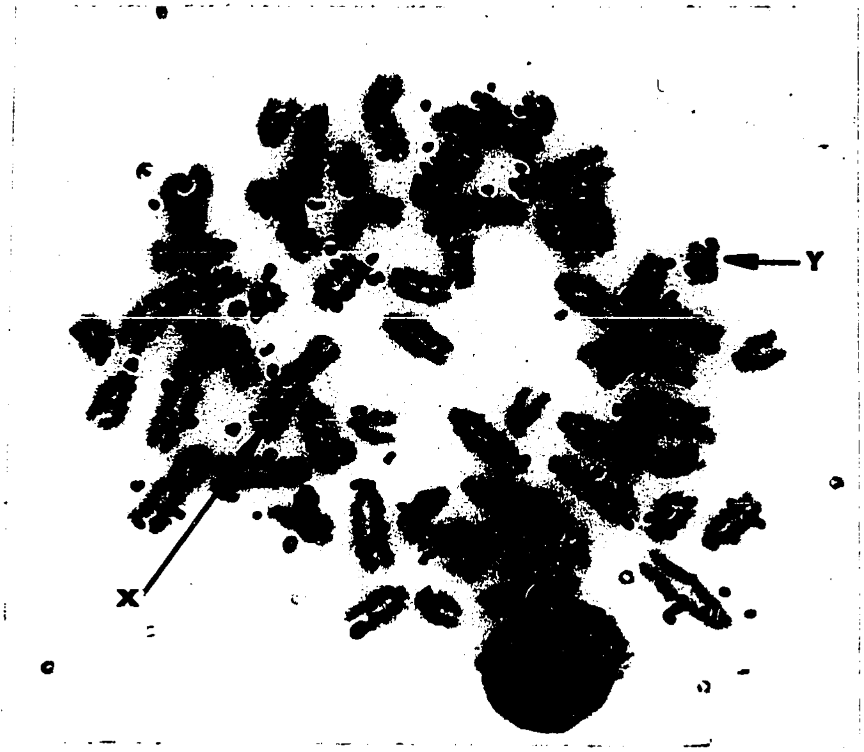


PLATE 6

Photograph and autoradiograph of a bovine male metaphase cell pulse labeled with H^3TdR during the mid S period (Table I, 5 hours after pulse, cell no. 11). The Y chromosome is one of the smallest chromosomes in the complement but could be identified by its submetacentric morphology. The X chromosome is the longest chromosome in the complement and is submetacentric. The autosomes are all acrocentric in morphology. The Y chromosome (Y) had 4 grains and the X chromosome (X) had 3 grains.


Y
X


Y
X

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