SPATIAL RELATIONSHIPS IN THE REPLICATION OF CHROMOSOMAL DNA

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Received January 11, 1965

THE chromosomes of cultured cells of the marsupial, *Potorous tridactylis*, have been described by WALEN and BROWN (1962) and SHAW and KROOTH (1964). The low number and the individuality of these chromosomes make this material particularly favorable for studies of chromosome duplication and segregation with tritiated thymidine techniques. There are ten autosomes and two X chromosomes in the female, and ten autosomes, one X and two Y chromosomes in the male. Studies with tritium-H^3 on several animal and plant cells have been reported since the initiation of chromosome labeling with tritium by TAYLOR, WOODS and HUGHES (1957), (TAYLOR 1963 review article; PRESCOTT and BENDER 1963; SCHMID 1963; PEACOCK 1963; MOORHEAD and DEFENDI 1963; GERMAN 1964). The results of these investigations may be summarized as follows: multiple sites for DNA synthesis occur along the chromosomes; whether or not homologous, sectors or whole chromosomes may replicate asynchronously as most clearly demonstrated for the sex chromosomes; both chromatids are labeled in the first metaphase after exposure to tritiated thymidine, whereas only one chromatid is labeled in later divisions; and, finally, labeling of both chromatids or sections in second metaphase (isolabeling) and substantial frequencies of sister chromatid exchanges may be observed. The present studies not only confirm these observations, but also present data which suggest that chromosome structure is responsible for a nonrandom distribution of old and newly synthesized DNA.

MATERIALS AND METHODS

Recently, detailed descriptions of autoradiographic techniques have been presented by several investigators (PERRY 1964; CARO 1964; PRESCOTT 1964; PRESCOTT and BENDER 1964; and SISKEN 1964). For the present study, therefore, only the immediate pertinent techniques will be described.

Cells were grown in Eagle's medium containing 4% fetal calf serum. Both bottle and coverslip cultures were used. Preparations from bottle cultures were made by the squash procedure, whereas coverslip cultures were air dried. The activity of the isotope used for pulse labeling was adjusted to 1 μc per ml of medium; the specific activity of the isotope ranged from 1.9 c/mmole to 6.7 c/mmole in the various experiments. Cells were exposed to the isotope for short intervals from 10 to 30 min, followed immediately by two washings with medium containing 100 times as much "cold" thymidine as the "hot" medium contained. The subsequent maintenance medium also contained the same concentration of "cold" thymidine. To determine the

This work was sponsored by the Office of Naval Research under the terms of a contract with the Regents of the University of California. Reproduction in whole or in part is permitted for any purpose of the United States Government.

generation time and any asynchronous replication of DNA, the population of cells was sampled every 2 hours for 70 hr; in subsequent experiments sampling was restricted to intervals yielding the maximum number of labeled first and second divisions. Each sample, except those for anaphase grain counts, was exposed to either colchicine (10^{-6} M) or colcemide (10^{-7} M) for periods varying from 2 to 8 hr immediately prior to standard treatment with hypotonic solution and fixation in Carnoy's solution. Both stripping (Kodak AR-10) and photosensitive emulsion (NTB-2) were initially used; the emulsion technique gave better results and was consequently used exclusively in later experiments. Slides were exposed for 5 days to 3 weeks, according to the specific activity of the isotope. Autoradiographs were developed in D-19b (1 to 3 min), and the cells were stained through the emulsion with azure-eosin Giemsa.

RESULTS

Confirmatory results: Several lines of tissue culture have been established from kidney cells of male and female animals. The populations remained largely diploid (Figure 1), although one line from a male contained an additional marker chromosome, which most probably originated through a pericentric inversion in the longest chromosome of the complement. Labeling trials with tritiated thymidine have shown that the duration of the mitotic cycle is 28 to 32 hr from metaphase one (M_1) to metaphase two (M_2). Early samples, 4 to 7 hr after isotope treatment, demonstrated asynchrony of DNA synthesis in the X chromosomes. In cultures from females, silver grains were present over the short arm of only one of the X chromosomes, while the rest of the complement was almost completely free of silver grains (Figure 2). Samples from later periods (14 to 23 hr) contained silver grains over all the chromosomes except the short arm of one of the X chromosomes (Figure 3). Identical sampling procedures with cultures from males resulted in the same type of asynchronous uptake of tritiated thymidine into the single X chromosome.

Figure 3 also shows that both chromatids are labeled in M_1, and grain counts on anaphase$_1$ figures showed a 1 to 1 distribution of labeled material. In general, one chromatid was labeled and one unlabeled in M_2, and sister chromatid exchanges were observed frequently. Isolabeling was also observed (Figure 4). The precise frequency of isolabeling was difficult to determine in these cells, since the chromatids usually stayed rather close together resulting in possible error due to the geometry of the chromatids, background labeling, or chance effects in the dispersion of a relatively small number of grains. However, there is no doubt that isolabeling does occur, and with considerable frequency.

New results: In two experiments relatively high frequencies of endoreduplication were observed. The process of endoreduplication was originally described by GEITLER (1939), who believed it to be a special type of endomitosis in which the chromosomes are duplicated twice without intervention of either mitotic or endomitotic chromosome movement. LEVAN and HAUSCHKA (1953) named this latter chromosome-doubling process endoreduplication. Thus, as a result of endoreduplication, the chromosomes appear in pairs in the subsequent mitotic metaphase (tetraploid) (Figure 5). In the present experiments, cells were found which had been labeled in either the first (ES_1) or the second (ES_2) synthetic phase. Discrimination between endoreduplication and other processes, such as endomitosis

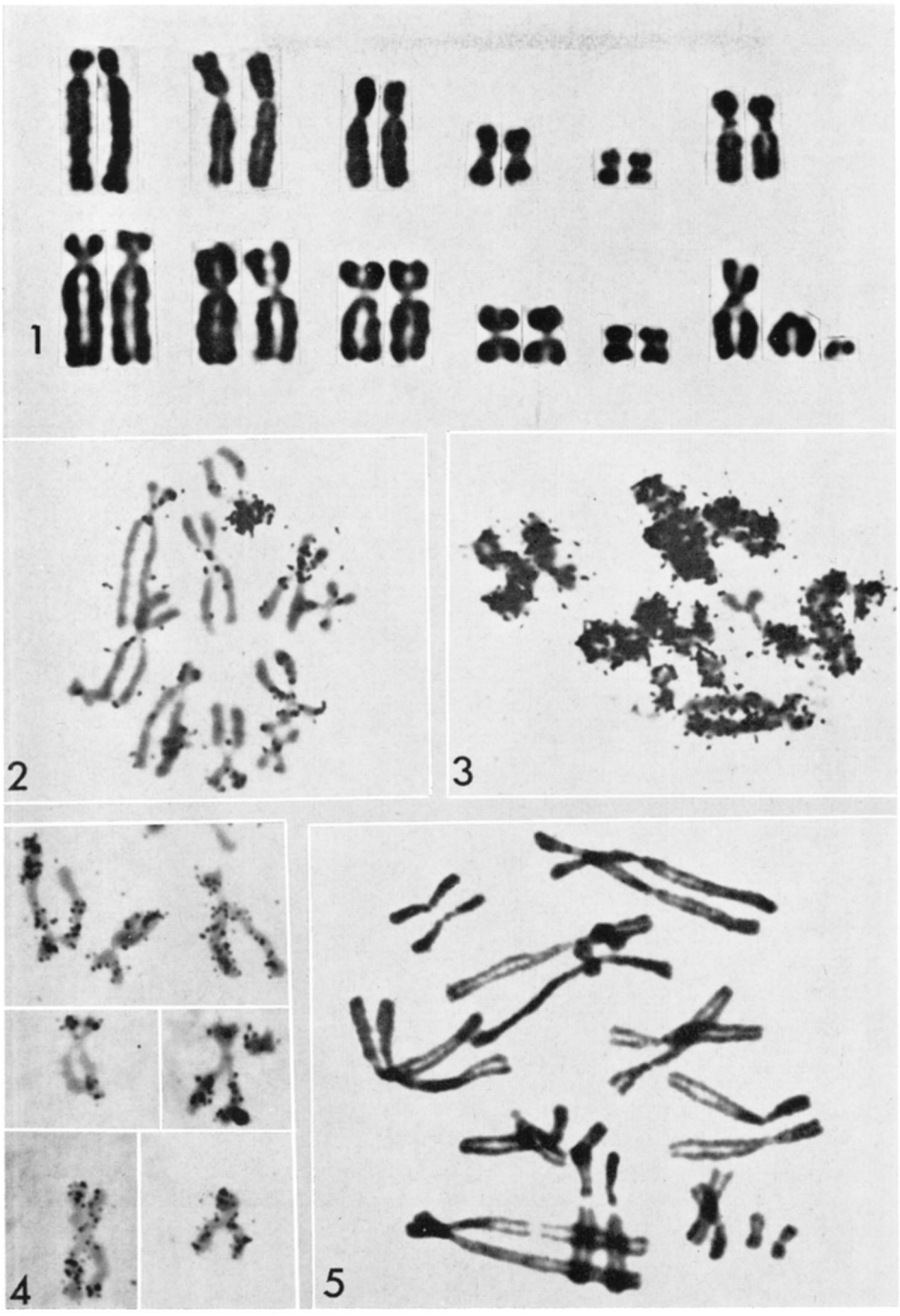


FIGURE 1.—The chromosome complement of *Potorous tridactylis*. Upper line female, lower line male; sex chromosomes to the far right.

FIGURES 2 and 3.—Labeled cells from early and late samples of a tritiated-thymidine treated cell population, showing asynchronous DNA replication of the short arm of one X chromosome.

FIGURE 4.—Examples of isolabeling over sister chromatids in the second division after labeling.

FIGURE 5.—An endoreduplicated cell showing the characteristic pairs of sister chromosomes.

and chromatid autonomy, will be considered in detail in the discussion. It should be noted at this point, however, that the observed results conformed almost completely to those expected from an endoreduplication sequence in which the pulse label had been incorporated at either ES_1 or ES_2 in a regular orthodox fashion.

Again, the cell population was sampled at intervals which normally would have yielded maximum number of labeled first (14 to 23 hr) and second (56 to 68 hr) divisions. Endoreduplicated pairs of chromosomes observed in the first sampling period contained silver grains over all four chromatids, whereas only two of the four chromatids were labeled in the second sampling period (Figure 6a,b). These observations are in conformity with those typically found in normal metaphases, namely labeling of both chromatids in M_1 and only of one chromatid in M_2. Figure 6 shows a cell with 11 pairs and only one chromosome of the 12th pair. Silver grains seem to be most heavily concentrated over the outer margins of several of the chromosome pairs rather than arranged at random with respect to the orientation of the chromosomes. Since the closeness of the chromatids (Figure 6) prevents an accurate grain count, an enlargement of the cell in Figure 6 was cut, and outer and inner chromatids were reassembled in pairs. In Figure 9, the pairs of the outer chromatids of Figure 6 are shown on the upper line and the corresponding inner pairs on the lower line. The last pair in each row of Figure 9 was reconstituted from the large twisted pair of chromosomes in Figure 6b by a second cut to compensate for the twist. It can be seen that over twice as many silver grains are present over the outer chromatids (upper row) as over the inner chromatids (lower row). Other examples of unquestionable labeling of the outer chromatids of one chromosome pair can be seen in Figure 7.

A total of 110 pairs of chromosomes were classified according to the arrangement of the labeled chromatids, and the occurrence of chromatid exchanges was also noted. A random relationship of the labeled chromatids would be expected to yield the following relationship: one outer-outer to two outer-inner to one inner-inner combination. It is also expected that endoreduplicated pairs of chromosomes will show both twin and single exchanges (TAYLOR 1958a). Only female material was used for tallying the chromosome arrangements in order that the chromosome complement could easily be divided into two groups: (1) eight large (L) chromosomes consisting of pair numbers 1, 2 and 3 plus two X chromosomes, and (2) four small (S) chromosomes consisting of pair numbers 4 and 5 (Table 1). In 20 cells examined, only 110 pairs of chromosomes were analyzable; in the remaining 130 pairs, the chromatid arrangements were unanalyzable because of overlapping, too few or too many grains, disruption of the pairs, or a combination of these factors. Among the 110 analyzable pairs. 47 were labelled only over the outer chromatids, only seven pairs showed an outer-inner combination of labeled chromatids; labeling of both inner chromatids was not observed.

In 56 cases, the arrangement of the chromatids was disrupted by exchanges, such that at least part of each pair showed an outer-inner arrangement. Since the small chromosomes, in which exchanges are infrequent, are largely of the outer-outer type (Table 1), it seems reasonable to conclude that an exchange

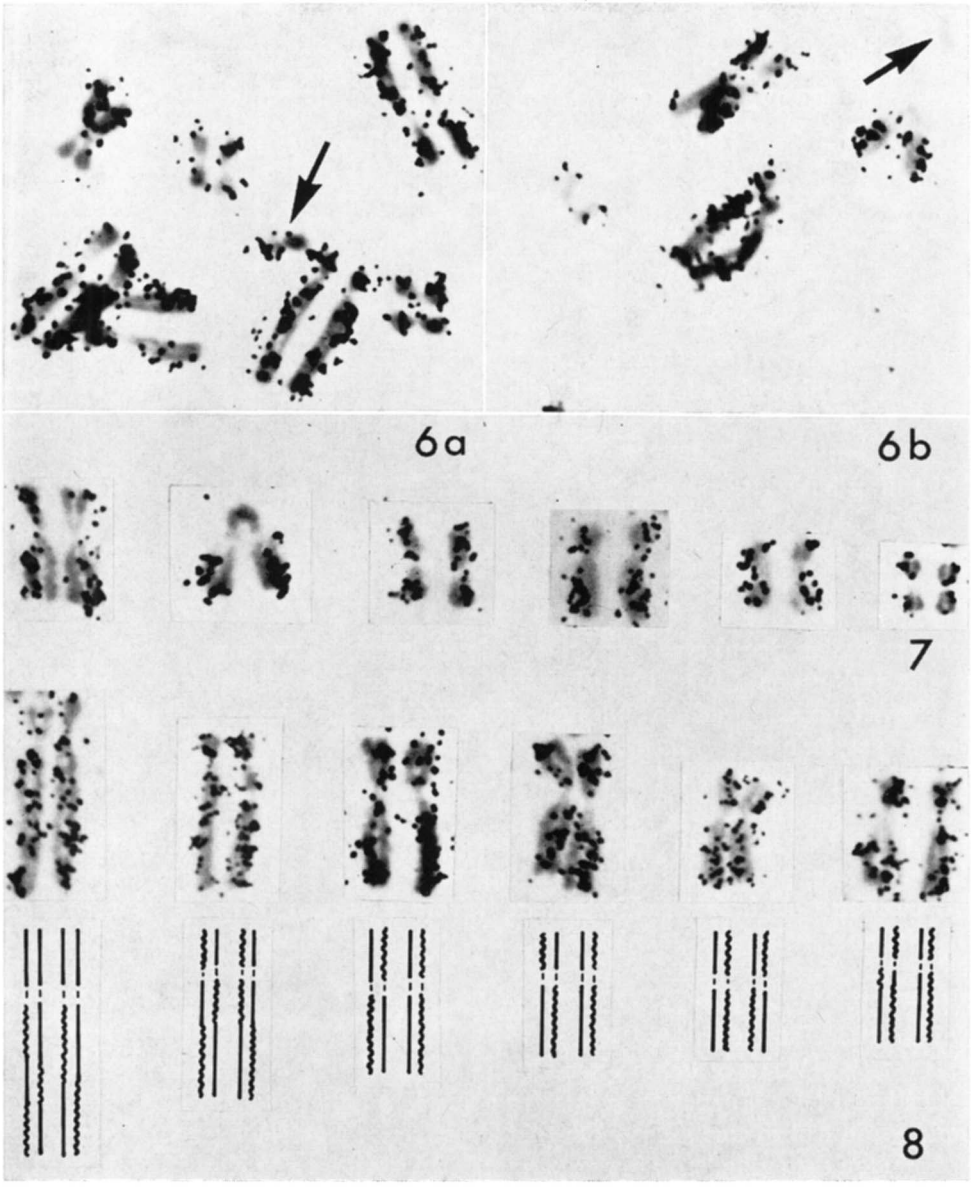


FIGURE 6.—(a and b) Labeled, endoreduplicated chromosomes, showing a concentration of the grains over the outer chromatids. The arrows point to one member and part of the other of the 12th pair of chromosomes (see text).

FIGURE 7.—Examples of labeling of the outer chromatids.

FIGURE 8.—Different types of sister-chromatid exchanges with the requisite crossovers below each pair. Wavy lines, labeled chromatids; straight lines, unlabeled chromatid.

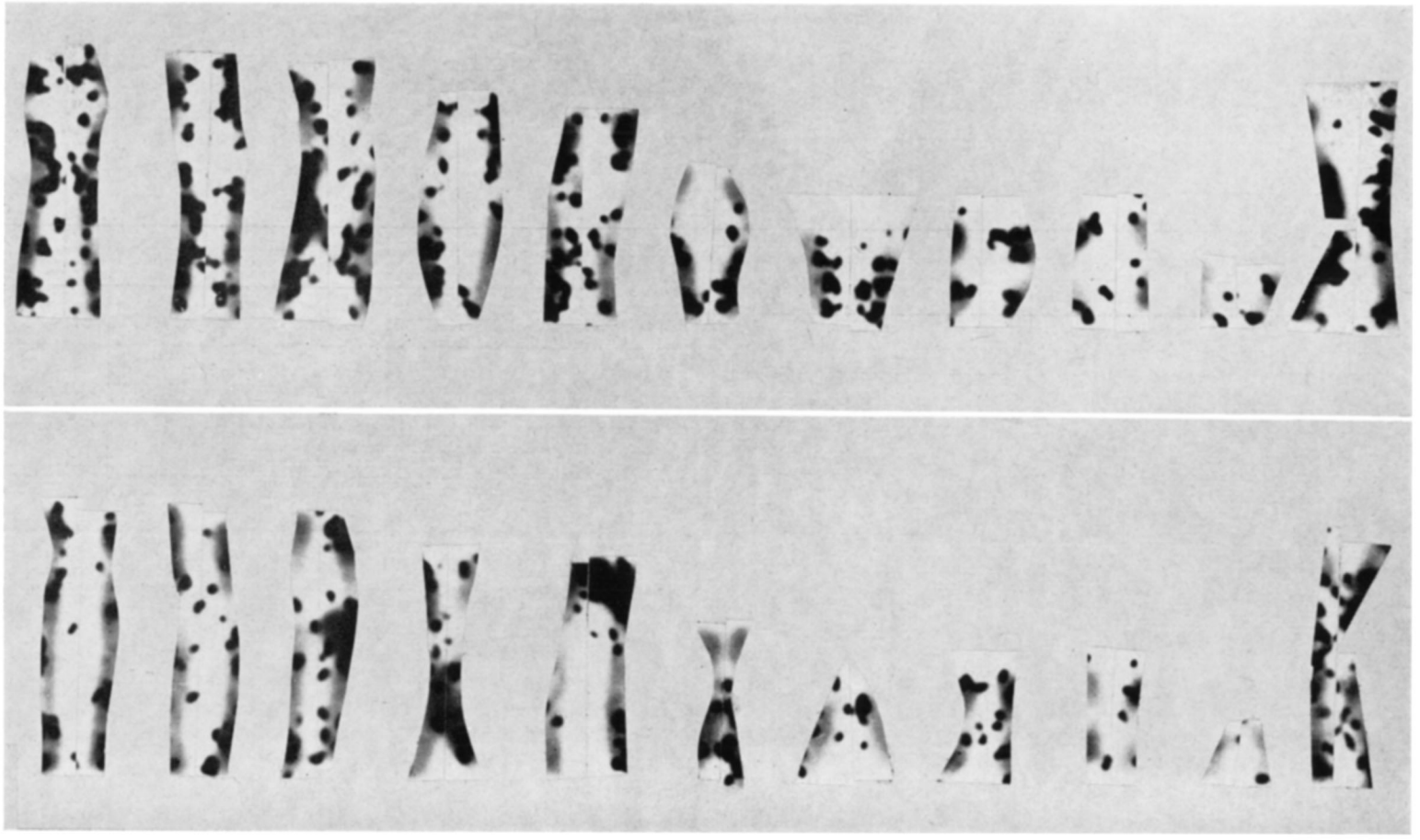


FIGURE 9.—The outer chromatids of Figure 6a and b on the upper line, and the corresponding inner chromatids of the chromosome pairs on the lower line.

had altered the pattern from what would have otherwise been an outer-outer relationship, rather than the converse. The different exchanges were classified into four types on the assumption that an outer-outer labeling pattern had been disrupted by either a single or a twin exchange, and these with their observed frequencies are illustrated in Figure 10. The different types of exchanges (Figure 8), and especially the fact that a great proportion of these occurred in the centromere region, will be considered in the discussion. Of a total of 56 pairs of chromosomes with sister chromatid exchanges, 41 pairs could be readily accounted for on the assumption that an outer-outer chromatid labeling pattern had been disrupted by the exchange. The remaining 15 pairs showed still further complications. In eight pairs, there were no silver grains over one or both short arms, whereas isolabeling occurred over one short arm in two other pairs. In the latter two pairs with isolabeling over one short arm, the outer chromatid of the other short arm was labeled; this would exclude the possibility that isolabeling here was due to an exchange between an inner chromatid of one chromosome and the outer chromatid of the other chromosome. The configurations in the last five pairs could be explained on the assumption that an exchange had altered an arrangement which would otherwise have been outer-inner. To summarize these data: of the 110 analyzable pairs of chromosomes, 88 pairs were classified in the outer-outer category, 12 pairs in the outer-inner, and 10 pairs were analyzable as far as the distribution of silver grains was concerned, but were not further classified for the reasons just given. These results clearly show a much higher frequency of outer-outer arrangements than would be expected on the basis of chance alone. Specific types of alterations expected from exchanges, as well as possible explanations for the occurrence of outer-inner combinations, will be considered in the discussion.

DISCUSSION

Confirmatory results: The chronology of the cell cycle, as revealed by labeling with tritiated thymidine, has been reported for a variety of cell populations (WIMBER 1960; TAYLOR 1960; PRESCOTT and BENDER 1963; EVANS and SCOTT 1964; SISKEN 1964). The labeling pattern of the X chromosomes of the marsupial, *Potorous tridactylis*, suggests that the short arm of one X chromosome replicates during the latter stages of DNA synthesis after the long arm and the entire other X chromosome have already been duplicated. A similar pattern of asynchronous synthesis, but with early rather than late labeling, has been reported for X chromosomes of female and male hamster cells (TAYLOR 1960). Labeling of both, or parts of both, chromatids in second metaphase (M_2) after exposure to the isotope, the so-called isolabeling, has been described in detail only for *Vicia faba* root cells by PEACOCK (1963), although such a labeling pattern has also been briefly noted by other investigators (TAYLOR 1958a; LA COUR and PELC 1958). Vicia root cells are more advantageous for determinations of the frequency of isolabeling than are the Potorous cells, because prolonged treatment of Vicia cells with colchicine leads to a complete separation of the chromatids (TAYLOR *et al.* 1957). Isolabeling observed in the present material is most probably not due to

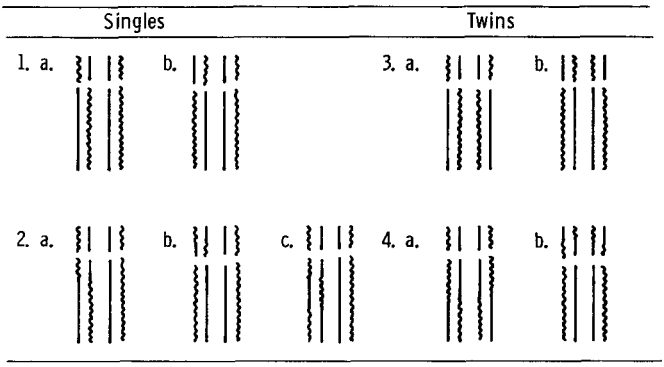
TABLE 1

Labeled chromatids of endoreduplicated pairs of chromosomes

Cell No.	Size class of chromosome pairs	No. pairs unanalyzed	No. pairs analyzed	Without exchanges* O/O	O/I	I/I	With exchanges No./chromosome pair Twin	Single
1	L	6	2	2	0	0	0	0
	S	3	1	1	0	0	0	0
2	L	5	3	0	0	0	1/1	3/2
	S	4	0	0	0	0	0	0
3	L	5	3	1	0	0	0	3/2
	S	3	1	1	0	0	0	0
4	L	7	1	0	1	0	0	0
	S	2	2	1	0	0	0	1/1
5	L	2	6	3	0	0	1/1	3/2
	S	0	4	2	0	0	0	2/2
6	L	3	5	1	0	0	1/1	7/3
	S	3	1	0	1	0	0	0
7	L	5	3	1	0	0	0	4/2
	S	2	2	2	0	0	0	0
8	L	4	4	2	1	0	0	2/1
	S	3	1	0	1	0	0	0
9	L	2	6	4	0	0	0	3/2
	S	0	4	4	0	0	0	0
10	L	3	5	1	0	0	1/1	4/3
	S	3	1	0	0	0	0	1/1
11	L	3	5	1	1	0	2/1	2/2
	S	2	2	1	0	0	1/1	0
12	L	4	4	1	1	0	0	2/2
	S	4	0	0	0	0	0	0
13	L	4	4	0	0	0	1/1	5/3
	S	3	1	1	0	0	0	0
14	L	2	6	3	0	0	1/1	2/2
	S	1	3	2	0	0	0	1/1
15	L	4	4	0	0	0	2/2	4/2
	S	2	2	2	0	0	0	0
16	L	5	3	0	0	0	1/1	2/2
	S	3	1	0	1	0	0	0
17	L	5	3	2	0	0	0	2/1
	S	3	1	1	0	0	0	0
18	L	5	3	0	0	0	0	3/3
	S	2	2	1	0	0	0	1/1
19	L	5	3	2	0	0	0	1/1
	S	2	2	2	0	0	0	0
20	L	3	5	1	0	0	3/2	4/2
	S	3	1	1	0	0	0	0
Total	L	82	78	25	4	0	14/12	57/37
	S	48	32	22	3	0	1/1	6/6
Total chromosomes		130	110	47	7	0	15/13	63/43

* O=outer chromatid; I=inner chromatid.

SISTER CHROMATID EXCHANGES



FREQUENCY OF EXCHANGES

Type			No. chromosome pairs
Single	1	Exchange in centromere for either long (a) or short (b) arm	12
	2	Exchange in either long (a) or short (b) arm and double exchange in long arm (c)	18
Twin	3	Exchange in centromere for either long (a) or short (b) arm	2
	4	Exchange in either the long (a) or short (b) arm	9
		Total	41

FIGURE 10.—Different types of exchanges and their frequencies on the assumption of a basic outer-outer chromatid labeling pattern. Wavy lines, labeled chromatids; straight lines, unlabeled chromatids; centromeres indicated by breaks.

residual isotope in the medium, since the pool of tritiated thymidine was diluted out by several washings with medium containing an excess amount of "cold" thymidine (TAYLOR 1958a; WIMBER 1960; PEACOCK 1963). Several other hypotheses have been offered by TAYLOR (1958b) to explain isolabeling. More recently PEACOCK (1963) has presented a semiconservative replication schema which explains isolabeling as the result of an occasional change in the normal pattern of distribution of strands from a bineme structure (two double helices). As pointed out by TAYLOR (1958b), however, multiple exchanges in short intervals could give rise to the appearance of nearly uniform labeling.

New results: Plant cells may undergo a further division after anaphase chromosome movement has been blocked by colchicine at the preceding metaphase; animal cells, however, do not usually respond in the same manner to such treatments. It should be mentioned, however, that LEVAN (1954) observed both endomitoses and endoreduplication after prolonged colchicine treatment of ascites tumor cells. In Potorous cultures in which endoreduplication had occurred, the cells had been exposed to colchicine for only 6 to 8 hours prior to fixation. In view of the rather long generation time, about 30 hr for these cells, it is very unlikely that colchicine treatment could have induced endoreduplication.

During the endoreduplication process, typical mitotic or endomitotic changes do not occur, so that if a mitotic division occurs subsequently a tetraploid division figure is produced in which the two sister chromosomes lie closely approximated in pairs. In endomitoses there is sufficient movement of the chromosomes during their contraction and elongation phase to cause considerable relocation of the chromosomes. In the example described by GEITLER (1937) of a 1024-ploid salivary-gland cell, produced by successive endomitoses, the 512 heterochromatic X chromosomes were dispersed throughout these giant branching nuclei. Likewise, in the experiments in which TAYLOR (1958b) blocked one division with colchicine, the chromosomes of the following tetraploid division figure were not associated in pairs. It appears that close pairing following endoreduplication may be explained on the assumption that there is little or no chromosome movement intervening between the two duplications.

Another process, presumed to account for an apparent doubling of chromosome number, is that of chromatid autonomy; in this instance, the chromosomes presumably fall apart without undergoing synthesis, and the chromatids behave thereafter as chromosomes. SCHRADER and HUGHES-SCHRADER (1958) used this explanation to account for identical amounts of DNA in both diploid and closely related tetraploid species.

Only chromatid autonomy immediately preceding the observed division could be expected to yield pairs of "chromosomes" (that is, chromatids masquerading as chromosomes)similar in aspect to those produced by endoreduplication. However, the labeling pattern expected from the two processes would be entirely different. If the chromosome pairs had been produced by a simple falling apart of pre-existing anaphase chromosome constituents (half chromatids), then the labeling would always be restricted to that acquired during the single synthesis in the sequence; thus, with the exception of isolabeling, never more than two of the four chromatids would be labeled.

The observed labeling of the four chromatids of one chromosome pair in the first sampling period is exactly analogous to the typical labeling of both chromatids in the usual M_1. The pairs of chromosomes, each with two chromatids, must therefore have been produced by a second round of DNA synthesis (ES_2). Thus, in the present experiments, the chromosome could be labeled in either the first or second duplication preceding the division figure revealing endoreduplication. It is only those that were labeled in the first division (ES_1) that are of concern here. Chromosome pairs produced by endoreduplication showed a preferential labeling of the two outer chromatids. Of the total of 110 analyzed pairs of chromosomes, 88 pairs (47 + 41, Table 1 and text) showed the outer-outer labeling pattern and only 12 pairs (7 + 5, Table 1 and text) fell into the category of an outer-inner labeling pattern. (See the preceding section for the ten pairs not classified). Labeling of both inner chromatids was not observed. The low number of outer-inner combinations and the total absence of inner-inner combinations may be explained as follows: all the endoreduplicated pairs of chromosomes are basically of an outer-outer labeling pattern, but, owing to a 180° turn of one chromosome during the flattening process of the cell, an outer-inner combination

would be the apparent result. For an inner-inner combination both chromosomes of one pair would have to turn 180°, which, by chance alone, was not included in the present, relatively small sample.

Regardless of the molecular organization or replication of DNA in the chromosomes, two rather simple assumptions may explain the nonrandomness of labeled chromatids within one endoreduplicated pair of chromosomes: (1) the centromere imposes a fixed geometric relationship between old and new DNA or, in other words, random segregation of old and new DNA does not occur in this chromosome region; and (2) some type of continuity of the old and new DNA exists throughout the individual arms of a chromatid such that a chromatid can consist of segments of both old and new DNA only after an exchange process. In other words, the morphological organization of the chromatid imposes a restriction on the spatial relationship between new and old DNA. In the following discussion, the functional replicating unit on the cellular level, rather than DNA synthesis on the molecular level, will be considered. The observed results are explicable then if the replicating units of a chromatid (anaphase chromosome) do not separate at the centromere in the ensuing interphase but are held together in such a manner that DNA synthesis cannot occur in between these units in the centromere region, and that, barring exchanges, continuity along the chromatid would maintain this relationship. This continuity could be achieved either through the longitudinal integrity of the entities involved, or the occurrence of further regulatory structures along the chromosome arms in addition to that presumed for the centromere. Recent suggestions on histone cross-linkages between DNA helices, such as that of ZUBAY (1964), obviously come to mind as possibilities for such regulation, but it would be premature to hypothesize a full-scale molecular model on the basis of the present unresolved and contradictory information on chromosome structure (see below).

To return to chromosome morphology, it should be mentioned that the centromere region is Feulgen positive, but less intensely so than the rest of the chromosome (LIMA-DE-FARIA 1956), and that tritiated thymidine autoradiographs show silver grains over this region. Considering that the centromere is the last region of the metaphase chromosome to divide, it seems very unlikely that an anaphase chromosome (chromatid) would divide again at the centromere during DNA synthesis in the interphase nucleus. The uniqueness of the centromere region in the cell cycle has been extensively discussed by DARLINGTON (1939, 1958), who, from analyses of misdivision of the centromere, advocated a single division of the centromere in late metaphase of mitoses. If, as the above evidence indicates, the replicating units do not separate, then the following interpretation of the spatial relationship of new and old DNA must be considered, namely, that DNA synthesis does not occur in between these units, but on the "outside". The first line of Figure 11 is an illustration of such a scheme for two normal cell divisions after exposure to tritiated thymidine. The basic labeling pattern in M_1 and M_2 is the same as predicted from other models on the molecular level (TAYLOR 1958b; FREESE 1958; SIMON 1961; PEACOCK 1963). On the second and third lines of Figure 11, the same replication scheme is applied to endoreduplication after the

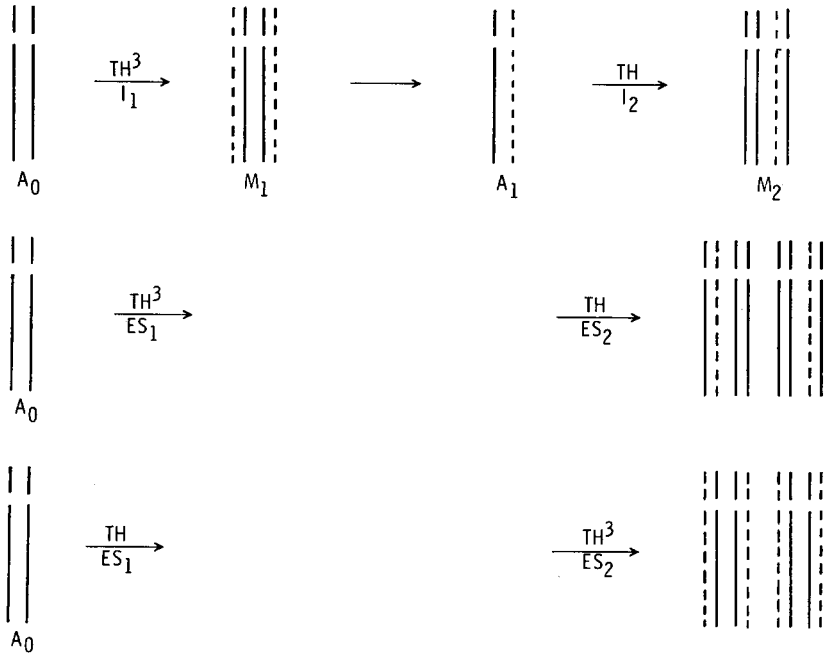


FIGURE 11.—Scheme showing replication of chromosomal strands. First line, expected labeling pattern in M_1 and M_2 after exposure to tritiated thymidine in I_1. Second and third line the same replication scheme applied to endoreduplication after exposure to the label in either the first (ES_1) or the second (ES_2) synthetic period. Straight line, no label; broken line, label; centromeres indicated by breaks.

presence of tritiated thymidine during either ES_1 or ES_2. It can be seen that presence of the isotope during the first synthetic period (ES_1) results in a labeling of only the two outer chromatids of an endoreduplicated pair of chromosomes, whereas all four chromatids contain label after an ES_2 synthetic period. As mentioned above, various sister chromatid exchanges and 180° turns of individual chromosomes on air drying of the cell could account for the observed disruption of the basic outer-outer chromatid labeling pattern. TAYLOR (1958a) discussed in detail single and twin exchanges and showed that colchicine affected the ratio of these exchanges, but this finding has since been disputed by PEACOCK (1963). Sister chromatid exchanges in the centromere region have been reported by MARIN and PRESCOTT (1964). They observed that one fifth of all second-cycle exchanges scored in M_3 occurred at the centromere. In the present material, this particular type of exchange could be scored in M_2 owing to the basic labeling pattern of the outer chromatids of an endoreduplicated pair of chromosomes. A twist in the centromere region would produce the same appearance as an exchange; however, the lack of flexures at the centromere in this material, implying a relatively rigid continuity here, implies that erroneous classification from this source has probably contributed little to the observed results. As can be seen from the diagrams of exchanges (Figure 10), the frequency of exchange in the centro-

mere region is also surprisingly high in the present material when compared to the rest of the chromosome. Somatic exchanges in Drosophila have also been shown to occur most frequently, if not almost exclusively, in heterochromatin (WALEN 1964), but whether the same basic mechanisms are responsible for the two types of exchange remains an open question.

The present scheme does not allow for discrimination between single-helix (unineme) semi-conservative and multihelix (polyneme) conservative replication. The latter replication scheme has, however, been excluded on the basis of density gradient studies of DNA from HeLa cells replicating in the presence of the 5-bromodeoxyuridine analogue (SIMON 1961). SIMON's data, on the contrary, show semi-conservative replication of DNA by the presence of a "hybrid" band after one division in the analogue and a "heavy" band after two divisions. Recently, various investigators have offered evidence for a polynemal structure of the chromosome (WILSON, SPARROW and POND 1959; KAUFMANN, GAY and MCDONALD 1960; RIS 1961; STEFFENSEN 1961; PEACOCK 1961, 1963). This contradictory evidence may be resolved by the *ad hoc* assumption that the semi-conservative replication of the chromosome is not a direct reflection of the semi-conservative replication of DNA itself, but of other, supra-molecular aspects of chromosome structure. Regardless of how this conflict may be resolved, the present evidence on the distribution pattern of old and new DNA imposes yet another restriction on models of chromosome replication.

I am very much indebted to DR. KAROL CONRAD for her many helpful suggestions and her assistance with certain technical aspects of this study. Special thanks and gratitude go to PROFESSOR SPENCER W. BROWN for many discussions and for help given during the preparation of this manuscript.

Note added in proof: In Chromosoma (Vol. **16** pp. 1–21, 1965) W. BEERMAN and C. PELLING have reported their results on labeling with H^3-thymidine in very early ontogeny of Chironomus. Autoradiographs of the salivary chromosomes made later in development show in many instances that the label is held in a single strand. Of significance here is the fact that the labeled strand usually occurs along the outer edge of the chromosome except in those instances where the strand appears to change from one side to the other which seems most likely to be due to twists in the chromosomes. The peripheral position of a primordial strand in the giant chromosomes clearly conforms to the results presented above.

SUMMARY

Cultured cells of the marsupial, *Potorous tridactylis*, were exposed to pulses of tritiated thymidine, and distribution of the label was observed over two mitotic divisions by grain counts over anaphase figures and by chromatid labeling in metaphase$_1$ and metaphase$_2$. In general, these results agreed with previously reported data on chromosome autoradiography, and in addition they provided evidences for a nonrandom distribution of old and new DNA. Thus, it was observed that endoreduplicated pairs of chromosomes contained the label in the outer chromatids when the isotope was present during the first synthetic period, whereas presence of the isotope during the second synthetic period resulted in labeling of all four chromatids. For the larger chromosomes of the complement, the basic outer-outer chromatid labeling pattern was frequently disrupted by

sister chromatid exchanges which often occurred in the centromere region. The preferential labeling of the outer chromatids of endoreduplicated pairs of chromosomes has a direct bearing on chromosome structure and replication.

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