left or right for 2 minutes at 10, 20, 30, and 40 degrees and with head upright (0 degree). The nine trials were presented in a different random order for each subject with a period of 4 minutes between each. The aftereffects for the nine angles of tilt are shown in Fig. 2, as are judgments of verticality during head-tilt. The latter, based on a separate group of ten subjects, were obtained under essentially the same conditions as the aftereffect except that adjustments to apparent verticality were made immediately after the head was tilted. It is clear from Fig. 2 that the visual effect during head-tilt was in the same direction as tilt, an effect referred to as the E-phenomenon (*7*), but that the visual effect following head-tilt (aftereffect) was opposite in direction. Tests of trend (*8*) applied to the aftereffect data indicated that the overall trends for both left and right head-tilt were significant ($p < .001$). Both linear and cubic components were also significant ($p < .01$). It is clear that, in addition to being opposite in direction to the E-phenomenon, the aftereffect increased with the angle of head-tilt and became maximum in the vicinity of 30 to 40 degrees.

The effect may be explained by apparent inclination of the upright head after protracted tilt. Some subjects reported that after the head was tilted to the right and returned to the upright position they felt as if the head were tilted to the left and vice versa. Ten subjects were required to adjust their heads to the upright after left or right head-tilt for 3 minutes at 30 degrees. All but two subjects exhibited an aftereffect of head position in the direction opposite to previous head-tilt. The mean aftereffect was 1.99 degrees. If the apparent tilt of the head was slightly opposite to the direction of previous prolonged tilt, the visual aftereffect could be the E-phenomenon resulting from apparent head position. But this explanation is doubtful since the apparent tilt of the head after 30 degrees inclination for 3 minutes was only 1.99 degrees. According to the E-phenomenon data shown in Fig. 2 this alone could not account for a visual effect as great as that found.

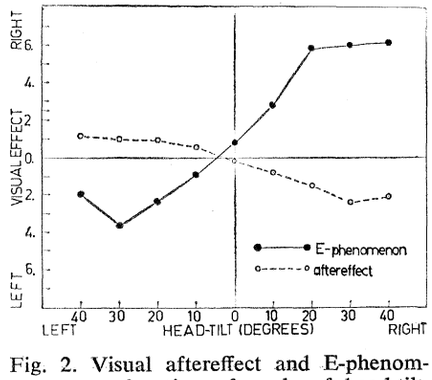

Fig. 2. Visual aftereffect and E-phenomenon as a function of angle of head-tilt.

The occurrence of a visual spatial aftereffect after prolonged head-tilt raises related questions concerning the mechanisms involved and the adequacy of current theories to explain the effect. Experiments (*9*) show that the aftereffect occurs when the observer is supine and the bar of light is directly above. This finding eliminates the possibility that the otolith system of the vestibular utricle, which is associated with the Aubert effect (*3*), is directly involved. Gravitational receptors of the vestibule do not respond to head- or body-tilt in the supine posture (*10*). One possible interpretation is that receptors in the joints and ligaments of the first three cervical vertebrae—these receptors affect the extraocular muscles (*11*)—are stimulated by lateral head-tilt.

Current theories which attribute visual spatial aftereffects solely to processes in the visual system (*1*) would require some modification to explain our data. A recent explanation proposed by Ganz (*12*), which treats spatial aftereffects as special instances of simultaneous illusions, would also require extension and modification to deal with a visual effect which does not derive from visual stimulation. Since the theory proposed by Gibson (*1*) is more general and makes no assumptions concerning the neurophysiological processes associated with aftereffects, it would have less difficulty in explaining these data. Both the visual and postural aftereffect from prolonged head-tilt can be attributed to Gibson's postulated changes in the norm of verticality. Clearly, then, the occurrence of an intermodal aftereffect requires some revisions of those theories which seek to explain spatial aftereffects.

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5. In adjusting the bar so that it appeared vertical, subjects set it in the same direction as previous head-tilt. It is inferred, therefore, that if the bar had remained vertical it would have been judged as slanted in the direction opposite to head-tilt.
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Segregation of Sister Chromatids in Mammalian Cells

Abstract. *Segregation of sister chromatids in embryonic mouse cells in primary tissue culture is not random. In mitosis those chromatids replicated on a DNA template synthesized during the preceding division cycle are separated from those constructed on a template synthesized two division cycles previously. Segregation in cells of the Chinese hamster follows a similar, but less pronounced, pattern.*

In bacteria, the products of chromosome replication, analogous to sister chromatids in higher organisms, are segregated into daughter cells in such a manner as to distinguish a chromatid containing a template strand synthesized in the previous division cycle from one containing a template strand synthesized in an earlier division cycle (*1*, *2*). This distinction between chromatids containing "parent" and "grandparent" templates has been explained with a model in which a polynucleotide strand attaches permanently to a subcellular segregation structure (membrane) when it is first used as a template in replication (*1*). When bacteria contain two linkage units or replicons, these segregate together. The replication products of chromosomes and episomes segregate so that units containing "grandparent" polynucleotide

templates are separated from those containing "parent" templates (*2, 3*). These latter results suggest that this mechanism of segregation might apply to chromosomes of higher organisms, and indeed our results indicate that in mice and hamsters this mechanism holds.

Primary tissue cultures were prepared from 18-day-old embryonic mice (*4*). Cells were grown in H^3-thymidine for 24, 48, or 72 hours corresponding to one, two, or three generations of growth (*5*). At these times, cells on cover slips were removed, washed, fixed in 3 percent gluteraldehyde, and dipped in Kodak NTB-2 emulsion. After a 4-day exposure, the preparations were developed, and the number of grains over each nucleus was counted (*6*). After one generation almost all of the cells incorporated the same amount of H^3-thymidine, corresponding to about 25 grains per cell (Fig. 1). After two generations, two classes of cells, with about equal numbers, were observed—one with about 25 grains per cell and one with about 50. After three generations, the same two classes of cells were still observed, but those with 25 grains accounted for only 25 percent of the population. Cells were also grown for one generation in radioactive medium and then for two generations in nonradioactive medium (Fig. 2). Cells on cover slips were again harvested after 24, 48, and 72 hours and analyzed by radioautography (radioautographs were exposed for 6 days). After one generation (that is, after a 24-hour exposure of H^3-thymidine) most of the cells had incorporated equal amounts of radioactive material (about 35 grains per cell in those autoradiographs exposed for 6 days). Cultures subsequently grown in unlabeled medium had two populations of cells, those containing about 35 grains per cell and those containing less than ten. The numbers of each were about equal after a 24-hour period of growth in nonradioactive medium. The proportion of unlabeled cells in cultures grown an additional 24 hours increased to about 67 percent. The average number of grains per cell in cells grown in nonradioactive medium decreased from 37 to 18 during the first 24-hour period and to 12 during the second, indicating that the cell number doubled during the first 24-hour period, increasing by another 50 percent during the second.

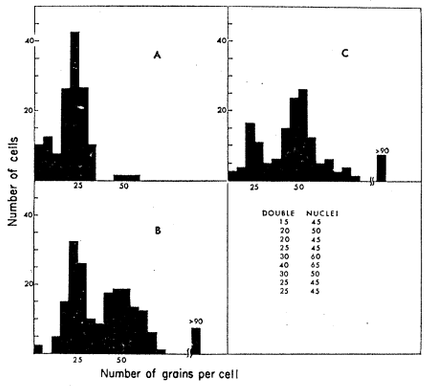

Fig. 1. Distribution of silver grains in autoradiographs of mouse embryo fibroblasts grown as a primary tissue culture for 1, 2, or 3 generations in H^3-thymidine (0.025 μc/ml) (*4*). *A*, An inoculum of 2.5×10^6 cells per petri dish (100 mm) was grown for 24 hours. *B*, 1.25×10^6 cells were grown for 48 hours, being replaced after 24 hours. *C*, 0.63×10^6 cells were grown for 72 hours, radioactive medium being replaced after 24 hours and 48 hours. Autoradiographs (*6*) were developed after a 4-day exposure. The number of cells examined was: *A*, 150; *B*, 200; *C*, 150. The numbers in the columns labeled "double nuclei" represent the numbers of grains in pairs of nuclei found in dividing cells in sample *B*. The two members of each pair appear opposite each other, and numbers have been approximated to the nearest multiple of 5.

Chromosome replication in mammalian cells is semiconservative (*7*). The results shown in Fig. 1 can be explained only if, at the second generation, those 40 chromatids (*8*) bearing two radioactive, conserved units are separated from the 40 containing an unlabeled, conserved unit. According to Fig. 2, those chromatids containing two nonradioactive units must be separated from those containing a radioactive one. Thus, in each cell, the chromatids containing a conserved unit or template made during the previous generation are separated from those containing a unit synthesized two or more generations earlier.

In the second generation of cells cultured as described in Figs. 1 and 2, a few pairs of cells still connected by cytoplasmic bridges were observed. Their nuclei, whose common ancestry can be assumed, reflect the overall behavior of the population; thus, the possibility that the two populations observed arise as a result of uneven rates of growth in different cells is eliminated. In Fig. 1 the population which is increasing becomes more radioactive, whereas in Fig. 2, an unlabeled population is increasing; thus, radiation does not select for or against the cell population.

In our studies we chose primary cultures of embryonic mouse tissue because: (i) The population of fibroblasts divides uniformly, and almost all of the cells become equally labeled within one generation. (ii) The cells use only 10 percent of the thymidine in the medium (0.2 to 0.4μg/ml). (iii) Mouse fibroblasts spend 70 percent of their division cycle in G_1 (*9*). Therefore, most of the cells contain one set of chromosomes that have not replicated, accounting for the sharp distribution in Fig. 1 and 2; few cells contain two sets of chromatids (cells in S or G_2 may have as much as twice the radioactivity observed in cells in G_1). (iv) We find that primary tissue culture cells of embryonic mice are synchronized with respect to DNA synthesis. In our experiments, most of the cells synthesize their DNA between 2 and 16 hours after the introduction of radioactivity (*10*). DNA synthesis is not appreciable until 10 hours after seeding of the cells. This also enhances the sharpness of the distributions in Figs. 1 and 2.

We thought the segregation mech-

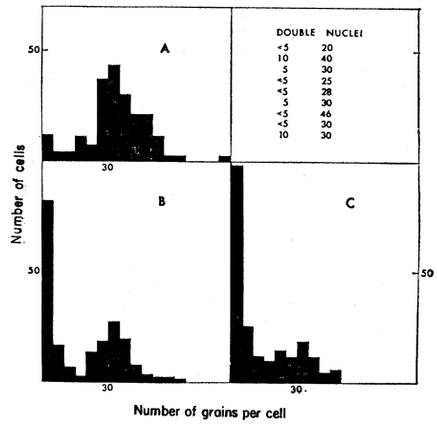

Fig. 2. Distribution of silver grains in autoradiographs of mouse embryo fibroblasts grown as a primary tissue culture for one generation in H^3-thymidine (0.025 μc/ml) and for two subsequent generations in nonradioactive medium (*4*). *A*, 0.3×10^6 cells per petri dish (35 mm) were grown for 24 hours, in medium containing H^3-thymidine. *B*, 0.15×10^6 cells were grown for 24 hours as in *A*, and then the medium was replaced with nonradioactive medium. *C*, 0.08×10^6 cells were grown for 24 hours as in *A* and then for 48 hours in nonradioactive medium. Autoradiographs (*6*) were developed after a 6-day exposure. Two hundred cells were counted for each population; the average number of grains per cell was *A*, 37; *B*, 18; and *C*, 12. As in Fig. 1, the columns labeled "double nuclei" represent the number of grains in paired nuclei found in dividing cells in sample *B*.

anism sought could best be observed on dividing diploid cells. As cells become polyploid, a segregation mechanism might be obscured by factors involving the distribution of extra sets of chromatids.

Cells of embryonic mice were labeled in primary tissue culture for three generations, treated with trypsin (*11*), and subcultured in nonradioactive medium for four more generations. The labeled and unlabeled populations of cells were not as well defined as those described in Fig. 2, and many cells with intermediate amounts of radioactivity were observed. The number of such cells was too great to be accounted for by sister chromatid exchange.

Figure 4 shows the results of a study similar to that in Fig. 2, but in which an established line of Chinese hamster cells (*12*) in which most of the cells are diploid was used. In these cells G_1 only occupies about 40 percent of the division cycle, and neither cell division nor DNA synthesis was synchronized upon subculture of the cells.

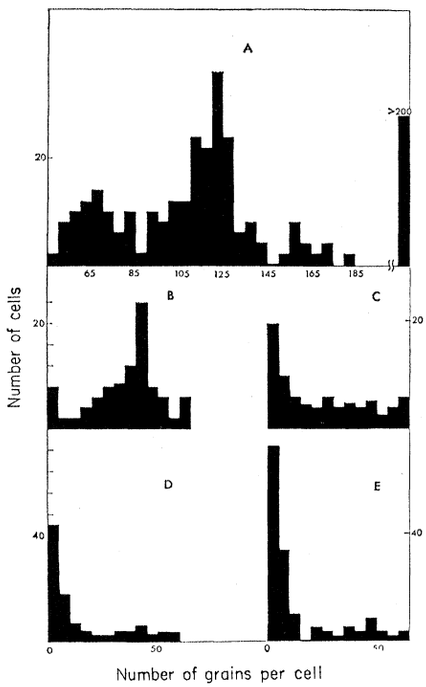

Fig. 3. Distribution of silver grains in autoradiographs of mouse embryo fibroblasts grown for three generations in .05 μc/ml H^3-thymidine and then transferred to nonradioactive medium for further growth (*4*). *A*, 0.63×10^6 cells grown in a petri dish (100 mm) for 72 hours in radioactive thymidine. They were then trypsinized and grown in nonradioactive medium. *B*, 2.5×10^6 cells were grown for 24 hours. *C*, 1.25×10^6 cells were grown for 48 hours. *D*, 0.62×10^6 cells were grown for 72 hours. *E*, 0.31×10^6 cells were grown for 96 hours. In *C*, *D*, and *E* the medium was changed every 24 hours. Autoradiographs (*6*) were developed after 4 days. The number of cells examined was *A*, 300, *B*, 100; *C*, 100; *D*, 100; and *E*, 160.

Previous experiments (*7*) indicated that in hamster cells, S plus G_2 lasted for about 60 to 65 percent of the division cycle. We found that a 30-minute pulse of H^3-thymidine labeled 55 percent of the cells; this finding indicates that 55 percent of the cell cycle was occupied by DNA synthesis (S).

After one and one-half generations of growth in radioactive medium, the distribution of radioactivity over the population is heterogeneous. This may be attributed to cells synthesizing DNA or having completed the synthesis of DNA prior to mitosis (that is, cells in S or G_2). When these cells divide in nonradioactive medium, the two radioactive chromatids can be distributed into daughter cells, all of which will then be labeled. Upon the next division in nonradioactive medium two cell populations are produced (Fig. 2). During this period the average number of grains dropped from 11.4 grains per cell to 4.6; thus the cell number increased by a factor of 2.5, and almost all of this increase is reflected by the presence of lightly labeled cells (0 to 5 grains per cell) which represent almost 60 percent of the population.

When correction is made for the background of 1 grain per nucleus (*13*) one can see that the lightly labeled cells comprise two populations. It is possible that the population containing a small amount of radioactivity (1 to 5 grains per cell) is a product of sister chromatid exchanges (*14*).

However, we would expect its distribution to be skewed toward low grain densities. The symmetry of the distribution suggests that in many cases one complete diploid set of sister chromatids is separated into a daughter cell which also receives a diploid set of unlabeled chromatids. This is supported by the fact that no distinct population of unlabeled or lightly labeled cells was observed in polyploid cells (KB and HeLa) labeled with H^3-thymidine for one generation and then grown for two generations in nonradioactive medium. However, the distribution is strongly biased toward the region of low grain counts.

Cells adapted to tissue culture show a tendency to segregate sister chromatids on the basis of the age of the template used in their synthesis (Figs. 3 and 4), but this effect is not as complete as it is in primary tissue culture. This may be the result of variations in ploidy arising during adaptation.

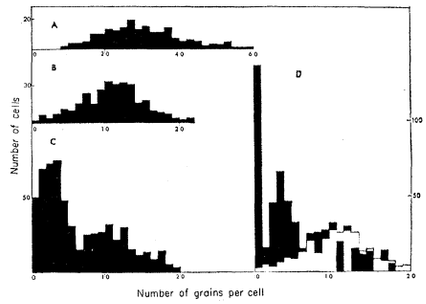

Fig. 4. Distribution of silver grains in autoradiographs of Chinese hamster cells grown for 24 hours in 0.1 μc/ml of H^3-thymidine and then for 48 hours in nonradioactive medium (*12*). *A*, 24 hours incubation with radioactive thymidine; *B*, 24 hours incubation with radioactive thymidine followed by 24 hours incubation in nonradioactive medium; *C*, 24 hours incubation with radioactive thymidine followed by 48 hours incubation in nonradioactive medium; and *D*, the distribution of grains in sample *C* corrected for background (*13*). The distribution in *B* is displayed in white on the corrected distribution for comparison. Autoradiographs (*6*) were developed after 4 days. The number of cells examined was: *A*, 200; *B*, 260; and *C*, 600. The average number of grains per cell was: *A*, 27.5; *B*, 11.4; and *C*, 4.6.

The simplest mechanism to explain the chromatid segregation is the one proposed for bacteria (*1*): When the conserved unit of a chromosome is used as a template for the first time, it is attached permanently to a structure distinct from that to which its parent chromosome was attached. As proposed by others (*15*), this structure could be the nuclear membrane. At division the new segregation structure and its attached chromatids would separate from the old. In this manner, each cell would always receive an exact complement of a diploid set of chromosomes at each mitotic division. Clearly this mechanism doesn't hold at meiosis when cells receive a haploid number of chromosomes selected at random from each diploid pair.

These results may help to explain the nonrandom distribution of chromosomal material observed by Whalen and by Feldman (*16*).

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4. The cells of 18-day-old embryos were seeded in plastic petri dishes containing cover slips (R. Consigli, H. Minocha, H. Abo-Ahmed, *J. Bact.*, in press). Unattached and dead cells were removed after eight hours. Cells labeled for 24 hours were therefore labeled in the period from 8 to 32 hours after seeding.
5. The specific activity of the H^3-thymidine was 5.8 c/mmole. Cells incorporated between 10 to 15 percent of the radioactivity in the medium during 24 hours of growth, after which time fresh, radioactive medium was added. Cells were seeded at different concentrations corresponding to the amount of growth desired. One generation of growth was calculated to be 24 hours.
6. See K. G. Lark and R. Bird (*1*). The radioautographs were stained with Giemsa. A background of between 1 to 2 grains per nucleus was *not* subtracted in the experiments described in Figs. 1, 2, and 3. Correction was made in Fig. 4 (*13*).
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10. At different times after seeding, the fraction of cells incorporating thymidine was: 10 hours, 8 percent; 14 to 18 hours, 60 percent; and 24 hours, 30 percent.
11. Petri dishes (100 mm) were seeded with 2.5×10^6 cells. After 72 hours of incubation with H^3-thymidine, the cells were washed three times with phosphate-buffered saline (PBS) and digested with 0.1 percent trypsin in PBS for 10 minutes. Trypsin was removed by centrifugation and the cells were used as inocula to seed the cultures described in Figs. 3*B*, *C*, *D*, and *E*.
12. The G-3 strain of Chinese hamster cells isolated from strain B14FAF28 was obtained from Dr. J. H. Taylor, who in turn received it from Dr. T. C. Hsu. It is primarily diploid [T. C. Hsu and C. E. Somers, *Proc. Nat. Acad. Sci. U.S.* **47**, 396 (1961)]. Cells were grown in Ham's medium [R. G. Ham, *Exp. Cell Res.* **29**, 515 (1963)]. In one generation (20 hours) 10^5 cells removed less than 10 percent of the thymidine from the medium. Radioactive medium was added to start the experiment 20 hours after cells were seeded. An inoculum of 6×10^5 cells per petri dish (100 mm) was used for all of the samples, regardless of the duration of growth.
13. Fig. 4*C* was partially corrected for background grains in Fig. 4*D*. The background was 1 grain per nucleus. The observed number of cells with no grains is the intersection of two probabilities—that of obtaining no grains due to background, 37 percent, and that of obtaining no grains produced by radioactive decay (which is thus the observed fraction of cells with no grains, divided by 0.37). From this we calculated the frequency of cells which had received one, two, three (and so forth) grains from background alone and subtracted these from the original distribution. No correction for background was made in the case of cells with one or more grains due to radioactive decay since the error is compounded by previous calculations.
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8 September 1966

Rapid and Marked Inhibition of Rat-Liver RNA Polymerase by Aflatoxin B_1

Abstract. *From 15 minutes to 2 hours after the administration of aflatoxin B_1 in vivo there is a 35- to 70-percent inhibition of DNA-directed RNA synthesis. The inhibition was reversed 12 and 24 hours later.*

Aflatoxin B_1 is a metabolite produced by certain strains of *Aspergillus flavus*, and its activity as a potent carcinogen for rat liver has been reviewed (*1*). Aflatoxin B_1 markedly inhibits hydrocortisone-induced synthesis of tryptophan pyrrolase (*2*). Other studies have shown that aflatoxin B_1 inhibits the incorporation in vivo of cytidine into rat-liver nuclear RNA and lowers the RNA content of the nucleus (*3*). In studying the mechanism of inhibition of enzyme induction and cytidine incorporation by aflatoxin B_1, we have found that soon after aflatoxin B_1 is administered there is a marked inhibition of DNA-dependent RNA polymerase in rat-liver nuclei. The inhibition was observed when 1 mg of aflatoxin per kilogram of body weight was administered, the same amount that prevents enzyme induction by hydrocortisone (*2*); but this dose is considerably lower than the LD_{50} (lethal dose, 50 percent effective) for the rat or the dose used to inhibit cytidine incorporation into RNA (*3*).

The activity of RNA polymerase was determined in nuclei isolated from livers of male Sprague-Dawley rats (200 g) that were fasted 20 to 24 hours before the experiment. Two animals per group were injected intraperitoneally with either 0.1 ml of triethylene glycol or 0.1 ml of triethylene glycol containing 0.2 mg of aflatoxin B_1 (extinction coefficient, E_{360}, is equal to 2.11×10^4 in methanol). Animals were killed by exsanguination 2 hours after aflatoxin treatment, the livers were rapidly removed and chilled, and nuclei were isolated from 8 g of liver by the method of Barondes, *et al.* (*4*), modified to include 0.002*M* $MgCl_2$ in all solutions. The nuclei were centrifuged (International T-60 ultracentrifuge) in 2.1*M* sucrose at 23,500 rev/min for 0.5 hour at 4°C. Nuclei were washed and brought to 2.0-ml volume with homogenizing sucrose. Preparations were assayed in duplicate at each interval, with 0.32*M* $(NH_4)_2SO_4$, 0.64*M* $(NH_4)_2SO_4$, and without any $(NH_4)_2SO_4$. The standard assay mixture for nuclear RNA polymerase was as follows: 100 μmole of tris, *p*H 8.0; 10 μmole of cysteine; 3 μmole of $MgCl_2$; 0.5 μmole each of adenosine, uridine, and guanosine triphosphates, respectively (ATP, UTP, GTP); 0.04 μmole of tritiated cytidine triphosphate (CTP-H^3) (2×10^7 count/min per micro-mole); 160 μmole of $(NH_4)_2SO_4$ and nuclei in a final volume of 0.5 ml. Each assay tube was incubated for 10 minutes at 37°C. Carrier RNA (2.0 mg) from yeast in 0.2*M* EDTA (ethylenediaminetetraacetate) *p*H 7.4 was added, and the reaction was immediately stopped with 2.5 ml of 10 percent perchloric acid which contained 40 μmole of sodium pyrophosphate and 10 μmole of EDTA. The mixture was allowed to stand 10 minutes and was then centrifuged at 2000 rev/min for 10 minutes. The pellet was successively washed: once with a mixture of 5 percent perchloric acid and 0.1*M* sodium pyrophosphate (4:1), twice with 5 percent perchloric acid, once with ethanol, and once with a mixture of ethanol, chloroform, and ether (2 : 2 : 1). The pellet was dried overnight. The precipitate was hydrolyzed with 1.0 ml of 5 percent perchloric acid at 90°C for 15 minutes; the hydrolyzate was cooled and centrifuged.

Table 1. RNA polymerase from liver of rats treated for 2 hours with aflatoxin B_1.

Additions to complete system		Deletions	Incorporation of CTP-H^3 (mμ mole/mg of DNA)	
Substance	Amt (μg)		Control	Treated
None		None	4.52	2.36
None		$(NH_4)_2SO_4$	2.00	.68
None		ATP,UTP,GTP	.24	.16
Deoxyribonuclease*	200	None	.24	.16
Ribonuclease	200	None	.36	.32

* Deoxyribonuclease was incubated with nuclei for 10 minutes at 37°C prior to the addition of CTP-H^3.