STUDIES ON THE MECHANISM REGULATING PERIODIC DNA SYNTHESIS IN SYNCHRONIZED CULTURES OF *ALCALIGENES FECALIS*

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(Received June 25th, 1960)

SUMMARY

Addition of deoxycytidine, deoxyguanosine, deoxyadenosine, and thymidine (10^{-4} *M* each) to the growth medium has been demonstrated to destroy periodic stepwise DNA synthesis in synchronous cultures. All four deoxyribosides are necessary. The effect is observed only if the deoxyribosides are added during DNA synthesis. It is accompanied by a loss of periodicity of RNA synthesis and of cell division.

At high medium concentrations (greater than 10^{-3} *M*), deoxyadenosine has been shown to selectively block DNA synthesis. This effect may be reversed by adenosine.

When a step of DNA synthesis—in a synchronous culture—is blocked, reversal of the inhibition results in resumption of DNA synthesis at that time when a second synthetic step occurs in cultures not treated with deoxyadenosine. This is true regardless of the time at which inhibition is initiated or reversed. This has been taken to suggest that DNA synthesis occurs only during certain "permitted" portions of the division cycle.

A hypothetical mechanism for controlling periodic DNA synthesis is discussed.

INTRODUCTION

Cultures of *Alcaligenes fecalis LB* in which the individual cell division cycles have been synchronized, show stepwise periodic synthesis of DNA and of a sedimentable RNA fraction[1]. In addition, the content of acid-soluble phosphorus within the cell changes in cycles whose period is the same as that found for nucleic acid synthesis and cell division[2]. Certain biological properties, such as the ability of the cell to concentrate intracellular metabolites and its susceptibility to penicillin, increase or decrease periodically[3,4]. On the other hand, synthesis of either cytoplasmic or cell wall protein is not periodic[3].

Several different systems for synchronizing bacteria have been described during the past few years[5]. In some of these[6,7] synthetic events occur in cycles, as in *Alcaligenes fecalis*; in others this is not the case[8,9]. Recent studies on DNA synthesis in single bacterial cells taken from exponential cultures have revealed, a lack of periodi-

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

city during their division cycle[10]. This lack of periodicity in single cells was shown to exist in the same organism which had exhibited stepwise DNA synthesis in synchronized cultures[6]. As such data have accumulated, it has become evident that bacteria—unlike cells of metazoan and some protozoan systems—do not display periodic synthesis of DNA as a necessary characteristic of the division cycle.

Cells of plant, animal, or protozoan origin show pronounced variations in the rates at which different compounds are synthesized during the division cycle. Regulation of the time of occurrence of intracellular events appears to be coordinated in a precise order[11–14].

What is the nature of the timing device which controls this coordination?

The demonstrated periodicity of several biochemically measurable parameters in the division cycle of *Alcaligenes fecalis* suggests this organism as a useful model for experimental approaches to this question.

In this investigation we have studied the control of periodic DNA synthesis. DNA was chosen because its periodicity could be easily observed and did not appear likely to be merely a reflection of some other periodic process within the cell. Previous studies had demonstrated that this synthetic periodicity was independent of both cytokinesis and gross protein synthesis[15].

Investigations of several systems have indicated that precursors of DNA may play an important role in the regulation of its synthesis. Studies on lily anther, on bacteria, and on tetrahymena have shown that deoxynucleosides or -tides accumulate prior to nuclear or cellular division[16–18]. Cells of *S. typhimurium* grown at different temperatures contain different quantities of acid soluble deoxyribosidic compounds. Upon shifting growing cells from a temperature of 25 to 37°, a sudden synthesis of DNA takes place which ceases as the quantity of these compounds is reduced[17]. Accumulation again occurs prior to the resumption of DNA synthesis.

In the present study, the addition of deoxyribosides to the growth medium has been used to interfere with periodic DNA synthesis. The results have made it clear that regulation of periodicity can occur at the precursor level.

MATERIALS AND METHODS

The methods for obtaining synchronized growth of *Alcaligenes fecalis LB* in a casamino acid medium have been described[3]. The methods used in the estimation of turbidity and cell number by the Coulter particle counter are also the same as previously published[3]. Measurement of DNA and RNA were carried out, as before, by the diphenylamine and orcinol reactions, respectively,[19,20]. Protein was estimated by the modified Folin reaction[21].

Samples for nucleic acid analysis were frozen until analyzed. Preparation of such samples for analysis followed the procedure already described[1] with the exception that samples from cultures treated with deoxynucleosides, particularly deoxyadenosine or -guanosine, were given three extra washes with cold 5 % TCA to remove all traces of deoxyribosides in the medium. In the majority of experiments deoxyadenosine was used as a comparative color standard for the diphenylamine reaction to obtain relative DNA values. Adenosine was used for the orcinol reaction. In obtaining the data given in Table I, standard sources of DNA and RNA were used to obtain absolute values.

Deoxyribosides used in these studies were obtained from the Sigma Chemical Company and were found to be chromatographically pure.

RESULTS

The DNA of *Alcaligenes fecalis* contains thymidine, deoxyguanosine, deoxyadenosine and deoxycytidine. When these deoxyribosides (10^{-4} *M*) are added to a synchronized culture, periodic synthesis of DNA is lost. This effect is shown in Fig. 1.

Other experiments have shown that the presence of *all four deoxyribosides* is necessary to achieve loss of periodicity. With the omission of any single deoxyriboside from the mixture, periodic DNA synthesis continues.

Figs. 2A and B illustrate the effect of adding deoxyribosides to a synchronous culture at different intervals. In the experiments in Fig. 2A samples of a synchronized culture were removed at 5, 10, 20, and 35 min and deoxyribosides added as in the previous experiment. DNA synthesis was followed in the control and in the four experimental aliquots of which only two showed a loss of periodicity. Those samples to which deoxyribosides were added before or after periods of rapid DNA synthesis were not affected. In sample 1 (deoxyribosides added before DNA synthesis), the

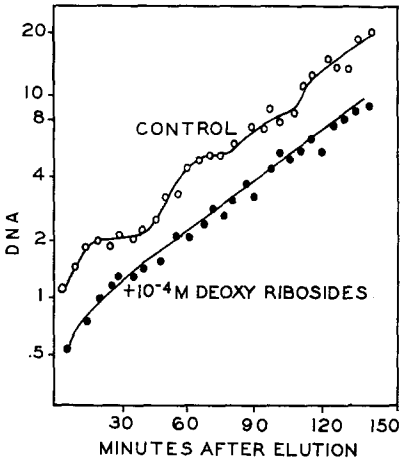

Fig. 1. Loss of periodic DNA synthesis upon addition of deoxyribosides. Deoxyadenosine, deoxyguanosine, thymidine, and deoxycytidine were added to a synchronized culture at 2 min after the experiment was begun. Each was added at a concentration of 10^{-4} *M*. Ordinates: DNA in relative units. Ordinate values for the curve with deoxyribosides were divided by 2.2 for clarity of presentation. Abscissa: Minutes after initiation of experiment by elution from filter paper. For the absolute quantities of DNA, RNA, and protein found in *Alcaligenes fecalis*, see Table I.

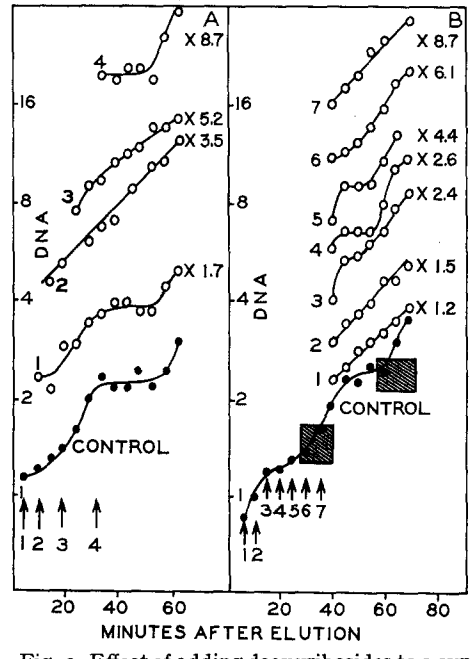

Fig. 2. Effect of adding deoxyribosides to a synchronized culture of *Alcaligenes fecalis* at different times. Times of addition of deoxyribosides are indicated by the arrows. Aliquots containing deoxyriboside have been multiplied by an arbitrary factor to enhance clarity of presentation. This factor is given at the right of each graph. Ordinates: DNA in relative units. Abscissa: Minutes after elution. The shaded area in (B) represents periods of cell division (cytokinesis).

continued presence of added deoxynucleosides did not lead to either an early burst of DNA synthesis or eventual loss of periodicity. Thus, there appears to be a crucial time at which deoxyribosides must be added to result in loss of periodicity. If added before this time, their continued presence does not exert an effect. This may be seen more clearly in Fig. 2B where deoxyribosides were added to a synchronous culture at different times during the first cycle of synthesis, and the effect on the second cycle was studied. Addition of deoxyribosides was effective only when it occurred at a time of DNA synthesis. Thus, periodicity was lost only in samples 1, 2, and 7 and partially disappeared in both 3 and 6. On the other hand, both samples 4 and 5 retained periodicity.

Control experiments have shown that cellular uptake of deoxyribosides does not significantly reduce their medium concentration during the course of the experiment. This is true regardless of the time at which they are added during the first cycle of DNA synthesis. Thus, the presence of deoxyribosides in the medium prior to the period of DNA synthesis results in a change in cell metabolism such that the cell is no longer affected by their presence during subsequent synthesis. A phosphorylase that splits thymidine to thymine plus deoxyribosephosphate has been described in *E. coli*[22]. Recent evidence has indicated that it may be induced within 10 min after the addition of thymidine to the medium[23]. A similar adaptation in *Alcaligenes fecalis* may account for the lack of effect of deoxyribosides added prior to the period of DNA synthesis.

Effect of medium deoxynucleosides on cell division and RNA synthesis

If periodicity in DNA synthesis is responsible in turn for periodic RNA synthesis and cell division, addition of deoxynucleosides should also induce asynchrony in these events.

To test the validity of this assumption, cell division and RNA synthesis were followed in the presence and absence of deoxynucleosides. A synchronized culture was split into two portions and, within a minute, deoxynucleosides were added to one of them. After 12 min each aliquot was again split in half and chloramphenicol added to one portion of each. As noted previously[15], the rate of RNA synthesis is reduced in the presence of chloramphenicol but this residual synthesis is stepwise. Therefore, addition of chloramphenicol facilitated observation of stepwise RNA synthesis. RNA, DNA and cell number were then followed in all four cultures: in medium alone, in the presence of deoxyribosides, in medium plus chloramphenicol, and in deoxyribosides plus chloramphenicol. The results of RNA and DNA analyses and cell counts on the four aliquots are shown in Fig. 3.

Loss of periodic DNA synthesis was accompanied by loss of synchrony in cell division (cytokinesis). In addition, the pattern of RNA synthesis was changed. Most striking in this respect was the increase in the early rate of RNA synthesis, both in the presence and absence of chloramphenicol. In both cases, the pattern of RNA synthesis clearly followed that of DNA synthesis. Loss of periodic DNA synthesis resulted in an increase in the amount of DNA in the culture during periods in which DNA synthesis ceased in the control cultures (see dotted lines in Fig. 3). Thus, an increase in the content of DNA appeared to result in an increased rate of RNA synthesis.

Inhibition of DNA synthesis by high concentrations of deoxyadenosine

In the course of experiments employing deoxyribosides at high concentrations, it was found that cells ceased to grow properly in the presence of deoxyadenosine in excess of 10^{-3} *M*. Cells developed into long filamentous forms and eventually ceased dividing. This effect was found only with deoxyadenosine and could be reversed by adenosine. Analysis of cells treated with deoxyadenosine revealed that a primary effect of this compound was on DNA synthesis. The nucleic acid composition of such cells is shown in Table I. Cells grown in deoxyadenosine plus adenosine were essentially identical in composition with those grown in plain medium, while those grown in deoxyadenosine alone had an abnormally low DNA content relative to RNA or protein. This finding indicated that deoxyadenosine treatment selectively blocked cell division and DNA synthesis, leading to a *larger* cell with relatively more RNA and protein than DNA. A similar effect has been described for ascites tumor cells[24].

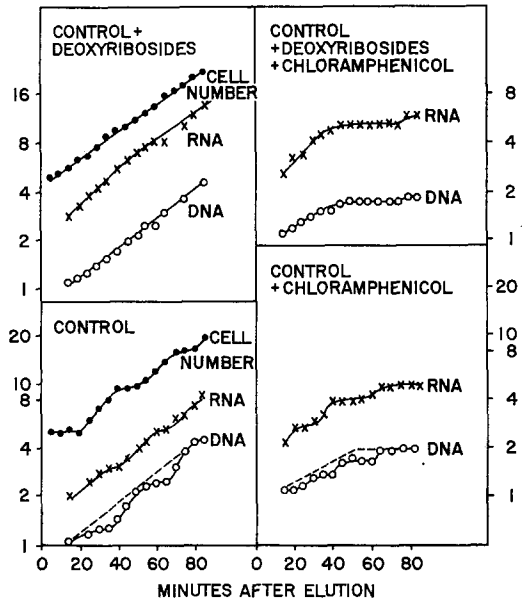

Fig. 3. Effect of loss of periodic DNA synthesis on RNA synthesis and increase in cell number. Deoxyribosides were added to a synchronized culture as in the experiment in Fig. 1. After 12 min, both the control and the deoxyriboside containing cultures were split in two portions, to one of which chloramphenicol was added. Values for DNA, RNA, and cell number in all four cultures are directly comparable. The dotted lines in the DNA graph in the control and the control-plus-chloroamphenicol cultures represent the course of DNA synthesis after the addition of deoxyribosides. Ordinates: DNA, RNA, and cell number in arbitrary units. Abscissa: Minutes after initiation of synchrony.

The time course of DNA inhibition by deoxyadenosine and the reversal of this inhibition by adenosine were studied in the following experiment. Deoxyadenosine was added to an exponentially growing culture at a concentration of $1.25 \cdot 10^{-3}$ *M* to initiate the experiment. At different intervals thereafter, samples were removed for analysis or cell count. At 40 (○), 120 (×), and 200 (△) min after the addition of

TABLE I

EFFECT OF DEOXYADENOSINE ON THE CHEMICAL COMPOSITION OF *Alcaligenes fecalis*

100 ml cultures were grown for 2 h in $1.25 \cdot 10^{-3}$ *M* deoxyadenosine (DOA), $1.25 \cdot 10^{-3}$ *M* deoxyadenosine plus $1.25 \cdot 10^{-3}$ *M* adenosine (DOA + A), and in normal medium (C). At the end of this time the average volumes per cell were: (DOA), 1.14 μ^3; (DOA + A), 0.67 μ^3; (C), 0.69 μ^3. Total cell numbers per 100 ml were: (DOA), $2.04 \cdot 10^{10}$ ml; (DOA + A), $3.84 \cdot 10^{10}$; (C), $4.56 \cdot 10^{10}$. Protein is given in equivalents of bovine serum albumin (Armour), RNA in equivalents of ribonucleate (California Foundation), DNA in equivalents of deoxyribonucleate (California Foundation).

	DOA 10^{-14} *g*	*DOA + A* 10^{-14} *g*	*C* 10^{-14} *g*
DNA per Cell	1.48	1.23	1.46
RNA per Cell	22.5	8.92	9.02
Protein per Cell	33.4	12.5	18.4

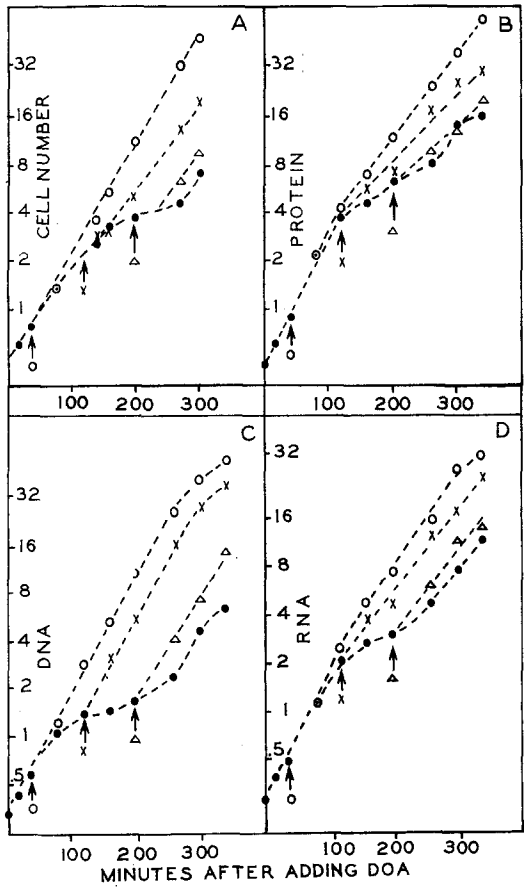

Fig. 4. Effect of deoxyadenosine on RNA, DNA, and protein synthesis and on increase in cell number. Deoxyadenosine was added at 0 min to an exponential culture at a concentration of $1.2 \cdot 10^{-3}$ *M*. Aliquots of the culture were diluted at intervals into adenosine. The intervals were 40 min (○), 120 min (×), and 200 min (△). Increase in cell number, protein, DNA, and RNA was followed in the original culture and in the aliquots to which adenosine was added. Ordinates: Cell number, protein, DNA, and RNA in arbitrary units. Abscissa: Minutes after adding deoxyadenosine.

deoxyadenosine, aliquots of the culture were diluted 3-fold into 10^{-3} *M* adenosine and these daughter cultures were also analyzed at intervals. The results are shown in Fig. 4.

For a short period after addition of deoxyadenosine, cell number and the amount of nucleic acids and protein continued to increase at the normal rate (*i.e.*, 40 min for a 2-fold increase). Subsequently, synthesis or division became slower and even ceased. After a further interval, the cells appeared to adapt themselves to the presence of deoxyadenosine and once again synthetic rates increased. Cell division and DNA synthesis were inhibited earlier than RNA or protein synthesis leading to cells with relatively high RNA and protein contents. After this occurred, all synthetic parameters appeared to behave similarly, although cell division remained comparatively retarded. As a result, cells became progressively larger although they preserved their new RNA: protein: DNA ratios. Such cells were viable and, indeed, cells will retain their viability in concentrations of deoxyadenosine as high as 10^{-2} *M*.

Addition of adenosine resulted in a reversal of the effects of deoxyadenosine. Resumption of cell division appeared to be delayed by one generation period in those instances where it had already ceased or was in the process of stopping. In contrast, the rates of all synthetic processes increased immediately relative to the deoxyadenosine culture from which the aliquots were taken.

It is natural to assume that when adenosine reverses inhibition of RNA, DNA, or protein synthesis, the cells which are in an unbalanced state (more protein and RNA than DNA) will attempt to revert as quickly as possible to their normal composition. To do this, the rate of DNA synthesis should exceed that of RNA or protein. This is what was found. *However, the rate of DNA synthesis did not exceed that found normally in exponentially growing cultures.* From the experiments[1] on stepwise synthesis of DNA (Figs. 1–3) it is known that under appropriate conditions (during a synthetic step for example) the rate of DNA synthesis can exceed the rate found in exponentially growing cultures. It is surprising, therefore, that in the experiment just described, cells recovering from inhibition of DNA synthesis did not synthesize DNA at a faster rate. This is especially true since the cells had a higher content of RNA and protein than usual.

The temporal control of DNA synthesis

Limitation of the recovery rate of DNA synthesis in the experiment in Fig. 4 would occur if DNA synthesis by individual cells was prohibited for certain periods. In such a case, periods in which synthesis of DNA was allowed would occur at random in an asynchronous culture as do the division cycles of individual cells. Temporal control of this process could be independent of the synthesis of DNA. Thus, upon adding adenosine to deoxyadenosine-inhibited cells, synthesis of DNA would be free to resume in each cell *as soon as an allowed period occurred.* In some cells this would be immediately; others could prohibit synthesis for as long a time as is *normally* required for a complete division cycle.

It was possible to test this hypothesis with synchronous cultures. Fig. 5 gives the results of an experiment in which a step of DNA synthesis was blocked, the block removed at different times, and the pattern of DNA synthesis measured. Deoxyadenosine was added to a synchronous culture at 20 min at a concentration of 10^{-2} *M*. This concentration of deoxyadenosine blocked protein and RNA synthesis

as well as DNA synthesis. Turbidity was used to estimate increases in mass, since the small amount of material available precluded reproducible direct analyses of protein. As may be seen in Fig. 5, synthesis of DNA and RNA, as well as increase in total mass, ceased following addition of 10^{-2} *M* deoxyadenosine. On the other hand, the increase in cell number continued, indicating that cytokinesis could proceed.

At 35 and 50 min, 15 and 30 min after the addition of deoxyadenosine, aliquots of the culture were diluted 6-fold out of deoxyadenosine into $1.5 \cdot 10^{-3}$ *M* adenosine. *Following reversal of the deoxyadenosine block, DNA synthesis did not occur until 65 min, regardless of when the block was reversed. This is the time at which a step of DNA synthesis occurred in the untreated synchronous culture.* In contrast, cytokinesis was delayed by a period equivalent to that for which the cells were in deoxyadenosine.

RNA synthesis commenced immediately upon reversal of the block but also showed periodicity which was independent of the time of addition of adenosine. Turbidity showed a behavior somewhat similar to that of DNA, although such a stepwise increase in mass was not found in synchronized cultures which had not been treated with deoxyadenosine.

The pattern of DNA synthesis shown in Fig. 5 was obtained in a large number

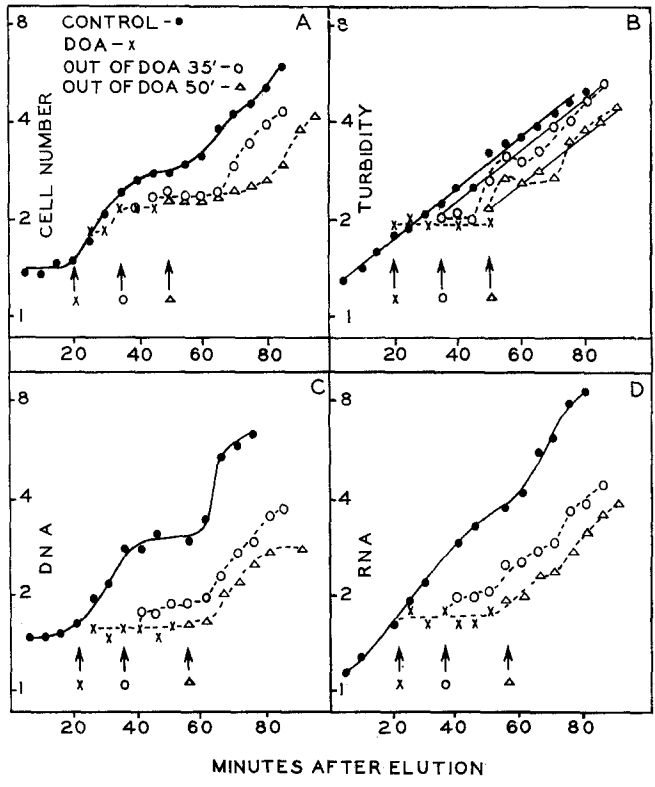

Fig. 5. Interruption of DNA synthesis by deoxyadenosine and the pattern of synthesis following removal of inhibition. Deoxyadenosine was added to the synchronized culture at 20 min at a concentration of 10^{-2} *M* (×). The culture was diluted out of deoxyadenosine into adenosine at 35 (○) and 50 (△) min. Cell number, turbidity, DNA, and RNA were measured. Ordinates: Cell number, turbidity, DNA, and RNA in arbitrary units. Abscissa: Minutes after elution.

of experiments and was the same when deoxyadenosine was added 20 min before the synthetic step, or when its effect was reversed at times other than 35 or 50 min. When the effect of deoxyadenosine was reversed during a period in which synthesis was taking place in the control (untreated) culture, residual synthesis occurred immediately. This has been seen repeatedly in experiments such as that shown in Fig. 5, *i.e.*, when deoxyadenosine was diluted at 35 min, a small burst (10%) of synthesis took place immediately. This did not happen in experiments where deoxyadenosine was diluted at 40 or 45 min.

When 10^{-2} *M* deoxyadenosine was added to an exponential, asynchronous culture, synthetic processes resumed immediately at the normal exponential rate following addition of adenosine. No periodicity was observed. However, a lag occurred before resumption of cell division.

From the data in Fig. 5 we can conclude that the periods between DNA synthetic steps are timed independently of the quantity of DNA per cell or of the occurrence of a previous burst of DNA synthesis. The data suggest that the proposed hypothesis of "allowed periods" of DNA synthesis is correct.

DISCUSSION

A discussion of the data may be conveniently divided into three portions:

(a) The relationship of RNA and DNA synthesis to the cell division cycle.

(b) Properties of the mechanism controlling the periodicity of DNA synthesis.

(c) Models of periodic control which are consistent with the data.

(a) *Relationship of RNA and DNA synthesis to the cell division cycle*

Previous experiments had indicated that synthesis of DNA occurs at about the same time as cell division. As more experiments have been carried out (over fifty during the past year) it has become apparent that DNA synthesis in synchronous cultures of *Alcaligenes fecalis* probably takes place immediately after cell separation. This conclusion is based on the observation that DNA synthesis frequently commences 5–10 min after cell separation (Fig. 3). This difference cannot always be seen, since an increase in cell number is often delayed because cells tend to stick together. This circumstance makes it difficult to pinpoint, with absolute certainly, a sequence of events relative to cell division.

RNA synthesis has been shown to be most rapid during and immediately following the period of DNA synthesis[1]. The division cycle of cells taken from synchronized cultures of *A. fecalis* may then be pictured (with the reservations mentioned above) as cytokinesis closely followed by DNA synthesis and rapid RNA synthesis.

In experiments in which synchronized DNA synthesis is deliberately destroyed, such as that shown in Fig. 3, synchrony of cytokinesis is invariably reduced. In the majority of cases, as in Fig. 3, it is almost completely absent.

This is consistent with the hypothesis that periodic increase in cell number reflects DNA synthesis and could be caused by the latter. However, it must be remembered that cytokinesis will also depend on other cell processes which may, under certain conditions, be limiting. An example of this is the delay caused by different periods of incubation in deoxyadenosine (Fig. 5) which blocks protein and RNA synthesis as well as DNA synthesis.

Periodicity of RNA synthesis is also lost when periodicity of DNA synthesis is destroyed. An explanation of this is suggested by the increase—upon adding deoxyriboside—in the rate of RNA synthesis in the presence or absence of chloramphenicol. Since loss of periodicity of DNA synthesis is characterized by an increase in the average content of DNA of the culture during the period in which DNA synthesis would normally be retarded, increases in DNA content may be responsible for increases in the rate of RNA synthesis.

This would explain the periodic synthesis of RNA which is normally observed in synchronous cultures[1]. Thus, every time a step of DNA synthesis occurs, RNA is synthesized more rapidly.

These data support the hypothesis that periodic DNA synthesis is basic to the cell and may be responsible for the periodicity of RNA synthesis or of cell division.

(b) *What are the properties of the mechanism controlling periodicity of DNA synthesis?*

(α) The regulatory mechanism is independent of the synthesis of DNA itself, *i.e.*, the synthesis is not autoregulatory. This follows from the experiment in Fig. 5 in which resumption of DNA synthesis was independent of the lack of a previous step of synthesis or of the period for which this synthesis was blocked. Since cytokinesis proceeded, these cells contained less DNA than normal. This finding implies that direct feedback or repression of DNA synthesis is not the mechanism whereby periodic synthesis is controlled, *i.e.*, net DNA synthesis is not limited solely by the amount of DNA per cell.

(β) Regulation of periodic synthesis can be controlled at the precursor level, since addition of deoxyribosides will destroy periodicity (Fig. 1). In the experiments described, all four deoxyribosides were required to achieve an effect. This implies physiological interference with DNA synthesis.

(γ) Destruction of periodicity at the precursor level only occurs during the period of DNA synthesis (Figs. 2A and B). Thus, it is only at this time that the regulatory mechanism becomes coupled with, or dependent on, DNA precursors. Precursors thus interfere with cessation or limitation of DNA synthesis rather than its onset or "trigger", since addition of precursors prior to DNA synthesis neither "triggers" the synthesis nor delays its onset.

(δ) the cell can adapt itself to the presence of added deoxyribosides in such a manner that the regulatory mechanism is not upset by their presence during DNA synthesis (Fig. 2B, Curves 4 and 5). This would indicate that mechanisms exist for either controlling the size of the intracellular pool or for bypassing it altogether. However, the cell requires advance warning to set up these mechanisms. As mentioned above, induction of specific enzymes which destroy deoxyribosides may play such a role*.

(ε) Following addition of deoxyribosides, periodicity of cell division is also lost. Since cytokinesis is a discontinuous event, this suggests that deoxyribosides are exerting their effect by randomizing the period between DNA synthesis in individual cells rather than by stimulating the continuous synthesis of DNA in all cells. This is only suggested by the data but appears to be a reasonable hypothesis which can be checked by autoradiographic techniques.

(c) *What models of periodic DNA synthesis can account for the data presented?*

Work in this laboratory has demonstrated that prior to a step of DNA synthesis

* See NOTE ADDED IN PROOF.

deoxyriboside containing materials are accumulated within the cell[17]. This increase is sudden and precisely timed indicating that it may be considered a discrete event. However, the amounts of acid soluble material involved are too small to be considered as a supply of precursor material for use in DNA synthesis. This finding indicated that the sudden appearance of precursor material may be catalytically involved in the initiation of DNA synthesis. Whether or not this is correct, the findings presented in this paper make it evident that the timing of DNA synthesis can also be controlled by the presence of deoxyribosides during DNA synthesis. As we have seen, neither the amount of DNA per cell nor the lack of consummation of its synthesis are important for the functioning of the timing mechanism.

What occurs during the period in which DNA may be synthesized which makes the timing mechanism so vulnerable to outside interference?

During replication of DNA, the molecule must uncoil to a sufficient extent to allow replication of sister strands. As a working hypothesis let us imagine that it is this separation of the DNA molecule which times the steps of DNA synthesis. (Such uncoiling and recoiling could even occur during deoxyadenosine inhibition.) Deoxyribosides could pair with bases on the separated strands, preventing recoiling and thus leading to additional DNA synthesis during the period in which strands are separated. Eventually the separated strands will again become intertwined and the cell will enter a period of no DNA synthesis.

Since, at best, a step of DNA synthesis is not perfectly synchronized in all cells and takes some time to accomplish, it is not unreasonable to expect that addition of deoxyribosides during a period of DNA synthesis catches individual cells in different stages of synthesis. This could prolong the synthetic period unequally for different cells. Once this has occurred, individual cells will be on different time schedules for DNA synthesis, leading to random total synthesis by the culture.

This hypothesis requires that the physical state of DNA has a profound influence on the timing of its synthesis. It demands that, in any cell, a certain period must elapse between the return of DNA to the native state and its ability to again enter the primer state.

It should be possible to test this hypothesis experimentally by means of autoradiography of single cells and by the study of the effect of deoxyribosides on the reversible denaturation of DNA.

NOTE ADDED IN PROOF

Since this paper was submitted for publication, evidence has been obtained in this laboratory that immediately upon addition of deoxynucleosides to the medium they appear in the intracellular pool, but disappear within 15 min further incubation.

(Received November 5th, 1960)

ACKNOWLEDGEMENTS

The author wishes to thank DR. M. MITCHISON of the University of Edinburgh, Edinburgh, Scotland, DR. D. M. PRESCOTT of the Oak Ridge National Laboratory, Oak Ridge, Tennessee, DR. R. W. SCHLESINGER of Saint Louis University School of Medicine, St. Louis, Mo., and DR. H. STERN of the Plant Research Division, Ottawa,

Canada, for helpful discussions during the course of this investigation or the writing of the manuscript.

Miss A. AGUADO and Mr. T. BARRY assisted the author in carrying out several of the experiments.

This work was supported by Grant E-1391 from the National Institutes of Health, Bethesda, Md., and, in part, by a grant from the National Science Foundation.

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