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## THE ROLE OF ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE IN THE REGULATION OF MAMMALIAN CELL DIVISION

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A number of investigations have implied in recent years that a relationship exists between adenosine 3',5'-cyclic monophosphate (cyclic AMP) and the growth of cells. Numerous experimental systems have been used to examine several different cell types. The result has been some definitive information about how cells divide, what role cyclic AMP plays in cell replication, and which periods of the cell cycle are affected by cyclic AMP.

This review article will primarily consider the role of cyclic AMP in mammalian cells in culture in both resting and dividing states. The examination of cultured cells has the advantage that the intracellular levels of cyclic AMP and the enzyme activities which may be influenced either directly or indirectly by this cyclic nucleotide can be examined as a function of a reasonably well-defined chemical medium. Furthermore, the role that cyclic AMP exerts in cells that have been shifted from the resting to the dividing state may be assessed. Because of the many different approaches that have been used, some of the information obtained appears to be contradictory (although it still suggests a role for cyclic AMP in cellular proliferation). The purpose of this report is to discuss individually the results that support specific aspects of the action of cyclic AMP at the morphological and molecular levels.

### IS CYCLIC AMP A MITOGEN?

The capacity of cyclic AMP to cause cells to convert or "transform" from a resting into a dividing

state must, by definition, be confined to a study of those cells that are physiologically repressed. Consequently, such investigations have been performed primarily in animal and human lymphocytes. These cells represent a unique system to study the mitogenic potential of cyclic AMP because they can be maintained *in vitro* in the resting state and will not undergo appreciable cellular DNA synthesis or mitotic division unless stimulated by a mitogenic substance (Nowell, 1960).

MacManus and Whitfield (1970) demonstrated that physiological concentrations of cyclic AMP ( $10^{-8}$ - $10^{-6}$  M) added to rat thymus cultures of lymphocytes stimulated DNA synthesis and mitotic division (see Table I). Additions of caffeine (0.8 mM), which presumably inhibits cyclic AMP phosphodiesterase under the conditions used, also increased the percentage of mitotic cells. Additions of cyclic AMP at concentrations greater than  $10^{-6}$  M, however, inhibited cell growth. These results *in vitro* were confirmed subsequently *in vivo* when a single intraperitoneal injection of cyclic AMP or its dibutyryl derivative in Colcemid-treated rats was shown to increase the number of bone marrow and thymus cells that entered mitosis (Rixon et al., 1970). The optimal dose of the cyclic nucleotides for this stimulatory activity was 5 mg/kg animal weight, and doses less than 2 mg/kg or more than 7 mg/kg produced essentially no activity. Since AMP-5' had no effect, the stimulatory action appeared to be a property

TABLE I  
Effects of Cyclic AMP on Nondividing Cells

Cell type	Additions (Concentrations)	Response	Reference
Thymocytes	Cyclic AMP ( $10^{-8}$ to $10^{-6}$ M) Cyclic AMP (Above $10^{-4}$ M)	Stimulated DNA synthesis Inhibited DNA synthesis	MacManus and Whitfield (1970)
Lymphocytes (porcine)	Dibutyryl cyclic AMP ( $10^{-5}$ - $10^{-4}$ M)	Stimulated DNA synthesis	Cross and Ord (1971)
Lymphocytes (human)	Cyclic AMP ( $10^{-6}$ - $10^{-5}$ M)	Slightly stimulated DNA synthesis	Hirschhorn et al. (1969)
Lymphocytes (horse)	Cyclic AMP ( $10^{-5}$ - $10^{-4}$ M) Cyclic AMP ( $10^{-5}$ - $10^{-4}$ M) Dibutyryl cyclic AMP ( $10^{-5}$ - $10^{-4}$ M)	No effect on DNA synthesis Stimulated RNA synthesis Inhibited RNA synthesis	Averner et al. (1972)

of this cyclic nucleotide. Furthermore, the concentrations of cyclic AMP that produce the appropriate mitotic response were found to approximate those that stimulated mitosis of thymic lymphocytes *in vitro*. Collectively, these results indicate that cyclic AMP is stimulating a cell that has already been programmed to undergo DNA synthesis and cell division. Thus, the role of cyclic AMP in thymocyte proliferation may be a promotional or auxiliary function rather than a mitogenic one.

Cross and Ord (1971) demonstrated that additions of *N*<sup>6</sup>, *O*<sup>2</sup>-dibutyryl cyclic AMP (dibutyryl cyclic AMP) at concentrations of  $10^{-5}$ - $10^{-4}$  M, stimulated DNA synthesis in porcine peripheral blood lymphocytes. The patterns of RNA and DNA synthesis, which were derived from the incorporation of labelled uridine into RNA and labelled thymidine into DNA, were identical to the patterns obtained when these cells were stimulated with phytohemagglutinin (PHA). Unfortunately, neither the nucleotide pool sizes, DNA content, nor mitotic indices were measured in these studies. Thus, the possibility exists that the rates of incorporation of labelled precursors do not accurately indicate the true synthetic rates for RNA and DNA. Interestingly, an increase in the size and specific radioactivity of the intracellular phosphate pool was observed after stimulation with both PHA and dibutyryl cyclic AMP. Also, an almost immediate increase in the specific radioactivity of phosphate incorporation into histone and histone kinase activity occurred within 30 min after stimulation. These data suggested to Cross and Ord (1971) that the activation of his-

tone kinase by cyclic AMP is one of the early events associated with the mitogenic stimulation of lymphocytes. Hirschhorn et al. (1969) observed that cyclic AMP (at concentrations of  $10^{-6}$ - $10^{-5}$  M) added to unstimulated human peripheral blood lymphocytes, produced a small but reproducible increase in DNA synthesis. The extent of synthesis as indicated by the incorporation of thymidine into DNA was only 2% of that observed with PHA. This result may indicate that cyclic AMP affects a cell type in the culture other than lymphocytes or, alternatively, a particularly small subpopulation of lymphocytes. They observed further that cyclic AMP, dibutyryl cyclic AMP, and theophylline in concentrations of  $10^{-4}$ - $10^{-3}$  M inhibited the DNA synthesis stimulated by PHA. In other studies (Johnson and Abell, 1970), additions of either dibutyryl cyclic AMP or cyclic AMP to unstimulated human peripheral blood lymphocytes from either normal donors or patients with chronic lymphocytic leukemia did not increase the rate of DNA synthesis.

Averner et al. (1972) demonstrated that cyclic AMP at concentrations of  $10^{-5}$ - $10^{-4}$  M stimulated the incorporation of labeled uridine into RNA but did not increase the incorporation of labeled thymidine into DNA in horse lymphocytes. Cyclic AMP increased the rate of RNA synthesis by 40-50% in these lymphocytes in either the absence or presence of PHA. Dibutyryl cyclic AMP, in contrast, inhibited RNA synthesis under these conditions. Averner et al. (1972) also found that cyclic AMP did not affect either the rate of uridine uptake into cells, the synthesis of uridine triphosphate, or the rate of degradation of RNA. Conse-

quently, they concluded that cyclic AMP had a direct effect upon the transcription but not replication of DNA.

#### THE EFFECT OF INCREASING CYCLIC AMP LEVELS ON CELL DIVISION

The intracellular level of cyclic AMP is determined primarily by adenylate cyclase, which catalyzes the synthesis of cyclic AMP from ATP, and cyclic AMP phosphodiesterase, which cleaves cyclic AMP to AMP-5' (Robison, 1972). The intracellular level of cyclic AMP may be readily increased either by stimulating adenylate cyclase activity, with the addition of a hormone or drug which mediates its action through cyclic AMP, or by the addition of an inhibitor of cyclic AMP phosphodiesterase such as a methylxanthine. Yet another method of increasing the intracellular levels of cyclic AMP is the addition of cyclic AMP or dibutyryl cyclic AMP to cells. In this method, it is presumed that cyclic AMP or dibutyryl cyclic AMP penetrates the cell membrane, thus directly increasing the intracellular levels of this cyclic nucleotide.

Studies in this laboratory (Abell et al., 1970; Johnson and Abell, 1970) have shown that additions of isoproterenol, dibutyryl cyclic AMP, and cyclic AMP greatly inhibit DNA synthesis in PHA-stimulated lymphocytes from patients with chronic lymphocytic leukemia at concentrations ( $10^{-5}$  M) that have essentially no effect on normal lymphocytes (See Table II). When higher concentrations ( $10^{-4}$ - $10^{-3}$  M) of these drugs were added, however, inhibition of DNA synthesis was observed also in normal lymphocytes. Studies by Smith et al. (1971a) demonstrated that isoproterenol elevated the cyclic AMP concentration above that observed in PHA stimulated cells and treatment with dibutyryl cyclic AMP at  $10^{-4}$  M concentrations resulted in pronounced inhibition of DNA synthesis (Smith et al., 1971 b).

Krug et al. (1972) found that resting lymphocytes, which contain only approximately six insulin binding sites, attain approximately 350 insulin binding sites on the cell surface within 24-48 h after stimulation with concanavalin A. With low concentrations, which stimulated DNA synthesis, it was further found that concanavalin A inhibited adenylate cyclase activity in broken cell preparations. Conversely, in high concentrations, concanavalin A inhibited DNA synthesis and

stimulated adenylate cyclase activity. These investigators suggested that it would be of particular interest to determine if the insulin receptors are the same as the membrane glycoproteins observed by Scott and Marchesi (1972), which appear on the surface of lymphocytes within 24 h after PHA addition.

Ryan and Heidrick (1968) found that cyclic AMP and dibutyryl cyclic AMP inhibited the growth of HeLa and L cells. Interestingly, dibutyryl cyclic AMP was less inhibitory than the parent nucleotide cyclic AMP. Cultures of human diploid fibroblasts as well as a number of lines of mouse fibroblasts have also been employed to study the effects of cyclic AMP, dibutyryl cyclic AMP, and theophylline on cell proliferation (Bürk, 1968; Johnson et al., 1971; Sheppard, 1971). These compounds inhibited cell growth and converted at least some of the morphological properties of virally transformed cells to normal phenotypes. Collectively, these studies strongly indicate that the elevation of the intracellular levels of cyclic AMP, induced either by stimulation of adenylate cyclase, inhibition of phosphodiesterase, or by cyclic AMP additions, inhibits cell growth.

#### INTRACELLULAR LEVELS OF CYCLIC AMP AND THE RATE OF DNA SYNTHESIS

The relationship between the intracellular levels of cyclic AMP and the rate of DNA synthesis (See Table III) has been examined in a number of cell lines (Otten et al., 1971). In studies performed on fibroblasts during logarithmic growth, they reasoned that if cyclic AMP inhibits the growth of fibroblasts, then slowly growing cells should have higher intracellular levels of cyclic AMP than rapidly growing cells. In a study of 13 different cell lines, it was found that the intracellular level of cyclic AMP was inversely proportional to the rate of DNA synthesis. Furthermore, when 3T3 cells were transformed with simian virus 40 (SV40), there was a corresponding decrease in cyclic AMP levels which correlated directly with increased growth rate of the cells.

Marks and Grimm (1972) determined the intracellular cyclic AMP levels of epidermis in mice that were maintained in a dark and light cycle. Cyclic AMP levels in the dorsal epidermis fluctuated with a pronounced diurnal rhythm, reaching a maximum during the resting phase and decreasing to a minimum during the activity phase of the

TABLE II  
Effects of Increasing Intracellular Levels of Cyclic AMP in Dividing Cells

Cell type	Additions (Concentrations)	Response	Reference
Lymphocytes (human)	Isoproterenol ( $10^{-4}$ - $10^{-3}$ M) Dibutyryl cyclic AMP ( $10^{-4}$ - $10^{-3}$ M) Cyclic AMP ( $10^{-4}$ - $10^{-3}$ M)	Inhibited DNA synthesis	Abell et al. (1970); Johnson and Abell (1970)
Lymphocytes (human)	Isoproterenol ( $10^{-5}$ - $10^{-3}$ M) Dibutyryl cyclic AMP ( $10^{-4}$ M) Aminophylline ( $10^{-5}$ - $10^{-3}$ M)	Inhibited DNA synthesis	Smith et al. (1971 a); Smith et al. (1971 b)
Lymphocytes (human leukemia)	Isoproterenol ( $10^{-5}$ M) Dibutyryl cyclic AMP ( $10^{-5}$ M) Cyclic AMP ( $10^{-5}$ M)	Inhibited DNA synthesis	Abell et al. (1970); Johnson and Abell (1970)
W138	Cyclic AMP ( $10^{-4}$ M)	Slightly inhibited cell growth	Heidrick and Ryan (1970)
HeLa, L, HEP2, and F1 amnion	Cyclic AMP ( $10^{-4}$ M)	Inhibited cell growth	Heidrick and Ryan (1970)
BHK 21/13, PyV, and BR7	Cyclic AMP ( $10^{-4}$ M)	Inhibited cell growth	Burk (1968)
Sarcoma and fibroblasts	Cyclic AMP ( $3 \times 10^{-3}$ M) Butyryl cyclic AMP ( $10^{-4}$ - $10^{-3}$ M) Dibutyryl cyclic AMP ( $10^{-4}$ - $10^{-3}$ M) Theophylline ( $10^{-3}$ M)	Restoration of phenotypically normal morphology	Johnson et al. (1971)
3T3 3T6 PyV-3T3	Dibutyryl cyclic AMP ( $10^{-5}$ - $10^{-3}$ M) Theophylline ( $10^{-3}$ M)	Restoration of contact-inhibited growth	Sheppard (1971)
Epidermal cells	Dibutyryl cyclic AMP ( $3$ - $5 \times 10^{-3}$ M) Theophylline ( $5 \times 10^{-3}$ M)	Inhibited cell growth	Voorhees and Duell (1971)

mice. Thus, high levels of cyclic AMP were found to coincide with low mitotic activity.

Sheppard (1972) also examined cyclic AMP levels in normal and transformed fibroblasts maintained *in vitro*. The steady state levels of cyclic AMP in normal density dependent fibroblasts were twice as high as those in a corresponding viral and a spontaneously transformed fibroblast culture. He also examined several components, including serum (Todaro et al., 1965), proteases (Burger, 1970; Sefton and Rubin, 1970), and in-

sulin (Temin, 1967), that influence growth and may affect the intracellular levels of cyclic AMP. A reduction of the intracellular cyclic AMP levels occurred after confluent monolayers of normal 3T3 cells were exposed to serum, trypsin, and insulin. Furthermore, prostaglandin  $E_1$  ( $PGE_1$ ) increased the intracellular cyclic AMP levels in cultured cells and transformed 3T3 cells.  $PGE_1$  also inhibited cellular growth in virally and spontaneously transformed cells (Sheppard, 1972). Thus, cyclic AMP levels and cell growth rate ap-

TABLE III  
*Relationship between Intracellular Cyclic AMP Levels and Cell Growth*

Cell type	Growth conditions	Growth rate	Intracellular cyclic AMP	Reference
Fibroblasts	Comparison of logarithmically growing cells	Increased	Decreased	Otten et al. (1971)
3T3	Transformed with SV40	Increased	Decreased	Otten et al. (1971)
Fibroblasts	Virally transformed	Increased	Decreased	Sheppard (1972)
Transformed cells	PGE1 addition	Decreased	Increased	Sheppard (1972)
L, HeLa, HEP2, F1 amnion, Ehrlich ascites, WI38, and fibro 5	Comparison of logarithmically growing cells	Decreased	Increased	Heidrick and Ryan (1971)
Diploid human fibroblasts	Contact-inhibited cells refed with fresh serum	Increased	Decreased	Froehlich and Rachmeler (1972)
3T3	Growth to quiescence	Decreased	Increased	Seifert and Paul (1972)

parently are altered or controlled by a number of different factors.

Heidrick and Ryan (1971) investigated the intracellular levels of cyclic AMP, cyclic AMP phosphodiesterase, and the rate of growth in several cell types including L, HeLa, HEP2, F1 amnion, Ehrlich ascites, WI38, and fibro 5. They found a direct relationship between the levels of cyclic AMP and the doubling time of these cells, but no correlation between phosphodiesterase activities and growth rate. Furthermore, two non-neoplastic cell lines had considerably higher levels of cyclic AMP than the transformed lines. Heidrick and Ryan (1971) suggested that the changes in cyclic AMP levels may be related to the phenomenon of cell contact inhibition. In another study by Froehlich and Rachmeler (1972), the levels of cyclic AMP were measured in human diploid fibroblasts. These cells, like the mouse fibroblast, demonstrate density dependent inhibition of cell growth. DNA synthesis in nonconfluent cultures and in contact-inhibited cultures, was stimulated by refeeding with fresh media and was found to be inhibited by exogenous cyclic AMP. Of more relevance, however, was the finding that the intracellular levels of cyclic AMP decreased when cells were stimulated to divide by adding fresh serum. These investigators concluded that their data supported the concept that cyclic AMP is related to the contact inhibition of cell proliferation. Although the mechanism for the inhibitory action of cyclic AMP is presently unknown, Froehlich and Rachmeler (1972) suggest that cyclic AMP may

function to control cell growth through the activation of a protein kinase. Substrates that are phosphorylated include such proteins as histones (Langan, 1971) and phosphorylase (Robison et al., 1971), as well as others (Kuo and Greengard, 1969). Although the physiological function of phosphorylation of proteins is not clearly understood at the present time, alterations of proteins in this fashion may contribute to the control of cell growth at the transcriptional level by affecting the RNA produced (as shown in a bacterial system by Pastan and Perlmann, 1972) or the translational level by regulating protein synthesis (Wicks, 1969; Barnett and Wicks, 1971; Walton et al., 1971).

An interesting report by Seifert and Paul (1972) compared the levels of cyclic AMP in sparse and dense cultures of both growing and quiescent 3T3 cells. In this study, 3T3 cells were cultured in medium containing 1% or 10% calf serum and were analyzed for cyclic AMP content during logarithmic growth and in the resting phase when the cells became quiescent. When cells were grown in 10% calf serum, they reached a saturation density of approximately  $10^7$  cells/dish, whereas when they were grown in 1% serum, they reached a saturation density of  $1.5 \times 10^5$  cells/dish. This result suggested that cell growth is dependent upon the amount of serum that is supplied to the medium. Interestingly, quiescent 3T3 cells, which were independent of cell-to-cell contact (grown in 1% serum) had an intracellular level of cyclic AMP that was twice that of growing 3T3 cells. When serum was added to quiescent cultures of

3T3 cells, a decrease in cyclic AMP levels was observed within 10 min, a finding which is in agreement with that observed by Sheppard (1971). Seifert and Paul (1972) concluded that the control of cyclic AMP levels in 3T3 cells is not affected by contact between cells and that serum factors are involved in their regulation. Apparently a serum growth factor is essential for 3T3 cells but is not required by transformed cells for growth (Paul et al., 1971). When this growth factor is depleted, cyclic AMP levels increase and the cells become quiescent.

#### CYCLIC AMP LEVELS IN THE STAGES OF THE MAMMALIAN CELL CYCLE

Smith et al. (1971 *a*), using a radioimmunoassay technique, found that intracellular cyclic AMP levels in normal lymphocytes increase significantly after the addition of PHA. Within 24 h, however, cyclic AMP was reduced to below baseline values. These results suggest that cyclic AMP may represent a signal for mitotic division and that an increase in the intracellular level of cyclic AMP is required early in the G<sub>1</sub> state of the cell cycle. In contrast, however, Novogrodsky and Katchalski (1970) found that PHA did not increase either the level of cyclic AMP or adenylate cyclase activity in rat lymph node lymphocytes. Also Makman (1971) demonstrated that PHA substantially decreased adenylate cyclase activity in homogenates of mouse and rat lymphoid tissues. In still another study, Hadden et al. (1972) found that additions of purified preparation of PHA and concanavalin A did not change the intracellular concentration of cyclic AMP in human peripheral blood lymphocytes, but did produce a 10- to 50-fold increase in cyclic GMP concentrations within 20 min after stimulation. They found further that small increases in cyclic AMP produced by the addition of PHA (similar to those observed by Smith et al., 1971 *a*) may be attributed to the agglutinating components in the preparation. They proposed that the increase in cyclic GMP levels may be a consequence of the response of cells to a mitogenic stimulant. Since these findings have not been investigated more extensively, the question of whether cyclic AMP or cyclic GMP is acting as an early messenger for the initiation of DNA synthesis still remains somewhat obscure. In our laboratory, we have found that 5 min after PHA is added to normal human lymphocytes,

intracellular cyclic AMP rises slightly and after 60 min, it is doubled. This level of cyclic AMP subsequently decreases within 24 h to below that observed in unstimulated cells and remains low during the replication of DNA (Monahan and Abell, unpublished observations).

In another study using synchronized HeLa cells, Zeilig et al. (1972) measured the concentration of cyclic AMP in each stage of the cell cycle. Specific fluctuations occurred throughout the cell cycle with the lowest levels of cyclic AMP reached during mitosis. Of particular interest, however, was the observation that when a specific inhibitor of cyclic AMP phosphodiesterase was added at the beginning of G<sub>2</sub>, mitotic division was delayed by 3 h. Millis et al. (1972) also observed that additions of dibutyl cyclic AMP caused a delay in mitosis in synchronized human lymphoid cells. In addition, Sheppard and Prescott (1972) have studied cyclic AMP levels in Chinese hamster ovary cells synchronized by mitotic shake-off. They observed that in comparison to the S stage, mitotic cells had a depressed cyclic AMP level, early G<sub>1</sub> cells had an elevated cyclic AMP level, and late G<sub>1</sub> cells possessed a cyclic AMP level similar to cells that were in DNA synthesis (S phase). With use of the isoleucine deficiency technique, which blocks cells in the G<sub>1</sub> period by the inhibition of protein synthesis, a depressed level of cyclic AMP was observed. These results suggest that protein synthesis is required for the establishment of appropriate levels of cyclic AMP. Furthermore, since adenylate cyclase is known to be more sensitive to hormone stimulation during mitosis (Makman and Klein, 1972), Sheppard and Prescott (1972) suggested that the changes in the cell surface during this period may also be accompanied by changes in the activities of enzymes associated with the plasma membrane. In conjunction with this change, the activity of cyclic nucleotide phosphodiesterase, which also influences the level of cyclic AMP, may fluctuate considerably over the cell cycle. Such determinations have not been performed in synchronized cells. Sheppard and Prescott (1972) also suggested that a decrease in cyclic AMP could lead to activation or synthesis of adenylate cyclase. Within this context, it would also be of interest to know the levels of cyclic GMP within the various phases of the cell cycle. As discussed above, Hadden et al. (1972) demonstrated that cyclic GMP concentrations are elevated within 20 min after PHA stimulation.

They suggest that this is an immediate signal produced by the mitogen which may, in fact, transfer information from the cell surface to the nucleus of the cell where DNA transcription occurs. Assays of cyclic GMP throughout the cell cycle may provide insight into the specificity and role of this cyclic nucleotide.

#### THE RELATIONSHIP BETWEEN INTRACELLULAR CYCLIC AMP LEVELS AND MALIGNANT CELL GROWTH

Numerous studies have been performed to establish a relationship between cyclic AMP and neoplasia. Agents which presumably increase intracellular cyclic AMP levels such as isoproterenol, dibutyryl cyclic AMP, and cyclic AMP greatly inhibit DNA synthesis in PHA stimulated lymphocytes from patients with chronic lymphocytic leukemia at concentrations which have essentially no effect on normal lymphocytes (Abell et al., 1970; Johnson and Abell, 1970). The inhibition obtained with dibutyryl cyclic AMP was essentially the same as that obtained with isoproterenol. Cyclic AMP produced less inhibition than either isoproterenol or dibutyryl cyclic AMP at the same concentrations. The inhibition obtained with all of the drugs was greater when they were added before DNA synthesis began but after the cells were shifted from a resting to a dividing state. This observation implies that an event in  $G_1$  is particularly sensitive to cyclic AMP. Furthermore, additions of theophylline potentiated the effects of isoproterenol. In other studies, Heidrick and Ryan (1970) have shown that cyclic AMP inhibited the growth of several cell lines (HeLa, L, HEP2, and F1 amnion) but had only a slight effect on one nonmalignant cell line (WI38). Cyclic GMP produced a similar inhibition of growth in both cell types.

As discussed previously, the direct relationship between cyclic AMP levels and cell doubling time (Goggins et al., 1972; Otten et al., 1971) has promoted the idea that at least some aspects of abnormal behavior of cultured transformed cells are due to the cells' inability to maintain normal amounts of this nucleotide. Other studies have also been performed in hepatoma cell lines (van Wijk et al., 1972). Some hepatomas transplanted in vivo have low adenylate cyclase activities which are insensitive to glucagon, whereas others are quite sensitive to this hormone and appear to be-

have like normal liver cells (Emmelot and Bos, 1971; Butcher et al., 1972). Unfortunately, adequate comparisons have not been possible because of the limitations of growing parenchymal cells continuously in culture. Furthermore, in most of the cell types that have been studied to date, measurements of all of the major determinants of the cyclic AMP system (adenylate cyclase, intracellular cyclic AMP levels, and cyclic AMP phosphodiesterase in the neoplastic state and in the cells or origin) have not yet been performed.

An interesting model for the study of cells that are rapidly growing but are not malignant is psoriasis. Voorhees and his co-workers (Voorhees and Duell, 1971; Powell et al., 1971; Duell et al., 1971) have found differences in adenylate cyclase activity between the fibroblasts obtained from normal donors and patients with psoriasis. Furthermore, additions of cyclic AMP reduce epidermal growth. These investigators suggest that psoriasis may be a "polygenic environmentally-induced defect in the adenylate cyclase AMP cascade" (Voorhees and Duell, 1971).

Taken together, all of these studies directly confirm or suggest that intracellular cyclic AMP levels are lower in malignant cells and that these cells are more sensitive to exogenously supplied cyclic AMP or to agents which stimulate intracellular levels of cyclic AMP.

#### CONCLUSION

From the foregoing discussion of studies that have been performed in many different laboratories, the following conclusions can be made.

- (a) Additions of cyclic AMP alone do not stimulate cells to divide except under unique circumstances in which cells, such as thymocytes, have already been programmed to undergo cell division.
- (b) Increases in cyclic AMP concentrations (induced either by stimulation of adenylate cyclase or inhibition of phosphodiesterase or cyclic AMP additions) in mitogen-stimulated lymphocytes inhibit cell division.
- (c) Intracellular levels of cyclic AMP and the rate of DNA synthesis are inversely related.
- (d) Cyclic AMP levels fluctuate throughout the cell cycle, but the question of whether mitogens induce immediate increases in this nucleotide has not been unequivocally resolved.
- (e) Cyclic GMP levels are rapidly and exten-

sively increased in mitogen-stimulated human lymphocytes.

- (f) Cyclic AMP inhibits malignant cell growth to a greater extent than normal cell growth.
- (g) A relationship between cyclic AMP and neoplasia cannot be unequivocally established at this time. However, since rapidly proliferating malignant cells generally have lower cyclic AMP levels and are more sensitive to inhibition by additions of cyclic AMP, a defect in the cyclic AMP system is a distinct possibility.

What must be considered now is the molecular site of action of cyclic AMP within the cell cycle. Cyclic AMP levels apparently are regulated precisely throughout the cell cycle. Relatively high levels (in comparison to those observed in mitosis) inhibit the progression of this cycle late in G<sub>1</sub>, S, and G<sub>2</sub>. The question of the role that cyclic AMP performs in controlling glycogen synthesis and degradation (Robison et al., 1971) microtubule formation (Goodman et al., 1970) and ribosomal RNA processing (Walton et al., 1971) in relationship to the cell cycle is currently not understood but these macromolecular processes may represent points of control. In fact, cyclic AMP may control DNA synthesis through other critical pathways which are prerequisites in a sequential manner for the synthesis of DNA and cell growth. The evidence appears to be clear that cyclic AMP exerts precise controls and is not merely toxic, causing a general cell death.

Another consideration is that cyclic AMP may play a dual role. For example, cyclic AMP could, at one point in the cell, stimulate phosphorylation of proteins that regulate the progression of the cycle but at a later point activate a phosphatase which in turn cleaves phosphate from the phosphorylated protein. Such a mechanism would provide for the control of replication through a common small nucleotide by stimulating at one point and causing inhibition at another. Although it is speculative at the present time, some evidence does exist for the role of cyclic AMP in controlling phosphatase in cells (Koyama et al., 1972 and De Lorenzo et al., 1973).

In conclusion, the information available strongly implies a role for cyclic AMP in the regulation of cell growth. The question of how this cyclic nucleotide exerts its influence upon critical cellular processes has only begun to be investigated.

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