CYCLIC AMP AND CYCLIC GMP[1]

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A chapter on "Cyclic AMP and Drug Action" has already been published in *Annual Review of Pharmacology* (1). In spite of its restrictive title, this paper covered most of the knowledge about the biochemical and biological effects of cAMP that was available in 1969. During the last four years, investigations of cAMP and cGMP have continued at an accelerated rate. Due to the restriction in the number of pages, the present review had to be selective and not exhaustive. We wanted to avoid a mere compilation of bibliographic references. The bibliography is therefore incomplete and only certain areas, which seemed to the author of special interest, have been described.

EFFECT OF cAMP ON SOME ENZYMES

Muscle phosphorylase has been most extensively studied. It exists in a form *b* (mol wt 185,000) that requires AMP for an allosteric activation and in a form *a* (mol wt 370,000) that is active without AMP. Activation is also effected by phosphorylation of some serine residues in phosphorylase b by a phosphorylase kinase which converts it to the a form (2). The phosphorylase kinase itself exists in a nonphosphorylated, inactive form, which is converted to a phosphorylated active form by the action of a cAMP-dependent protein kinase which might be called phosphorylase kinase kinase (3). This enzyme can also use as substrates other proteins like casein and protamine. The role of cAMP has been recently elucidated (4–7). cAMP-dependent protein kinases are composed of a catalytic subunit C and of a regulatory subunit

[1]Abbreviations: cAMP (adenosine 3',5'-monophosphate); db-cAMP (N^6,2'-O-dibutyryl adenosine 3',5'-monophosphate); N^6-mb-cAMP (N^6-monobutyryl adenosine 3',5'-monophosphate); 2'-O-mb-cAMP (2'-O-monobutyryl adenosine 3',5'-monophosphate); cGMP (guanosine 3',5'-monophosphate); db-cGMP (N^2,2'-O-dibutyryl guanosine 3',5'-monophosphate); RO-201724 [4-(3-butoxy-4-methoxybenzyl)-2-imidazolidone]; SC-2964 (1-methyl-3-isobutylxanthine); SO-20009 [1-ethyl-4-(isopropylidenehydrazine-1H-pyrazolo-3,4)-pyridine-5-carboxylic acid ethyl ester]; GH (growth hormone); TSH (thyroid stimulating hormone); mRNA (messenger ribonucleic acid); tRNA (transfer ribonucleic acid); UDPG (uridine diphosphoglucose).

R. cAMP binds to R; this promotes the dissociation of the enzyme according to the equation

$$\underset{\text{inactive complex}}{\text{cAMP} + \text{C–R}} \rightleftharpoons \text{R–cAMP} + \underset{\text{active form}}{\text{C}}$$

In addition, a protein inhibitor I of these enzymes has been discovered in a wide range of mammalian tissues (8, 9). It lowers the initial velocities; the interaction of I is noncompetitive with ATP and casein. The factor I does not catalyze the dephosphorylation of the phosphoprotein produced and its action is independent of the nature of some of the protein substrates. Among several possible modes of action, the following one could be demonstrated (10):

$$\begin{aligned} \text{R–C} + \text{cAMP} &\rightleftharpoons \text{C} + \text{R–cAMP} \\ \text{C} + \text{I} &\rightleftharpoons \underset{\text{inactive form}}{\text{C–I}} \end{aligned}$$

The catalytic unit C must therefore be free before the reaction with I that leads to its inactivation. In other tissues (liver, adrenal cortical tissue, and brain), the mechanism of activation of phosphorylase may be different.

Glycogen synthetase (UDPG-glycogen transglucosidase) exists in the skeletal muscle in two forms: I and D. The former is independent of glucose-6-P for activity, while the latter is dependent on this cofactor (11). The conversion of I to D involves a phosphorylation by a cAMP-dependent synthetase kinase (12–14). On the other hand, the conversion of D to I (activation) is a dephosphorylation catalyzed by a relatively specific phosphatase (12, 15). More recently, phosphorylase kinase kinase and synthetase kinase were shown to be the same enzyme (16, 17). cAMP activates the breakdown of glycogen by stimulating phosphorylase, but inhibits the synthesis of the polysaccharide by inactivating glycogen synthetase.

Similar cAMP-dependent protein kinases were found in many other tissues. In certain cases, cAMP, 5'-AMP, and 5'-ADP activate phosphofructokinases; however, the existence of a cAMP-dependent kinase that would convert the enzyme to an active form by phosphorylation is uncertain. An allosteric effect has not been ruled out; it has been suggested that cAMP, AMP, and ADP compete with ATP, which is inhibitory, for an allosteric site of phosphofructokinase (18). Triglyceride lipase is stimulated by cAMP, which actually activates a kinase that phosphorylates the lipase and converts it to a so-called active hormone-sensitive lipase. This explains the lipolytic effect of epinephrine and its antagonism by insulin and prostaglandins which decrease cAMP levels in fat pads (19).

One general theory proposed regarding the mechanism of action of cAMP involves modifications of Ca^{2+} levels and an increase in the permeability of cell membranes to Ca^{2+} (20). Another theory involves activation of protein kinases by cAMP (21). Although the second representation rests, in certain cases, on a firm experimental basis, it seems premature to reduce to this common denominator the multitude of effects of cAMP.

CYCLIC GMP

cGMP is the only cyclic nucleotide besides cAMP that is known with certitude to occur in nature. This compound was discovered in urine as radioactive phosphoorganic compound after injection of inorganic ^{32}P into rats (22). Assay methods have been worked out (23–26) that allow a more detailed study of cGMP, which has been detected in all mammalian tissues investigated so far, as well as in several lower phyla. In most tissues the concentrations of cGMP are generally at least tenfold lower than those of cAMP. For instance, in various rat tissues, the amounts of cGMP are between 10^{-8} and 10^{-7} mol/kg of wet tissue (24–26). Hormones that increase cAMP levels do not elevate cGMP levels; theophylline does, however, increase these levels in some tissues. In plasma, and especially in urine, large amounts of cGMP and cAMP are excreted; in rat urine the daily excretion is several times higher than the amounts of cyclic nucleotides contained in a given time in the whole body. The excretions of cAMP and cGMP are generally controlled independently by hormonal effects. This fact suggests the existence of a specific enzyme system, guanyl cyclase, which catalyzes the synthesis of cGMP and uses GTP as substrate. In contrast to adenyl cyclase, it is soluble in most tissues of the rat and has been partially purified from lung and liver (27, 28); it appears to be mostly particulate in the rat small intestine (29). The richest source has been found in the sperm of the sea urchin, where the levels are up to 1000 times higher than in rat tissues; there the enzyme is entirely particulate (30). Detergents like 1% Triton solubilized or dispersed the particulate form and enhanced its apparent activity (29, 30). The question of the formation of pyrophosphate in addition to cGMP from GTP, and the question of the reversibility of the reaction, are still open. Guanyl cyclase is almost absolutely dependent on Mn^{2+}, which cannot be replaced by Mg^{2+} or by Ca^{2+}. However, in the presence of low amounts of Mn^{2+}, Ca^{2+} produces a strong stimulation. In contrast to adenyl cyclase, guanyl cyclase is not activated by fluoride in broken cell preparations, and the activity in cell free systems from liver and heart is unaffected by the hormones so far tested: glucagon (27), insulin (27), epinephrine (27), ACTH (31), thyroxine, and cortisol.

In broken cell preparations, cGMP is generally inactive or much less active than cAMP (32). Concerning the activation of kinases, cGMP in high amounts produces in some cases the same maximum stimulation as cAMP. However, half maximal activation of the enzymes required concentration of cGMP about 100 times higher with respect to rat liver glycogen synthetase kinase (33) or to the synthetase activity of a protein kinase from rabbit skeletal muscle (17). cGMP has been found virtually without effect on a cAMP-dependent protein kinase from adipose tissue (34) or from heart and skeletal muscle (35). An interesting exception was seen with a preparation of protein kinase from lobster muscle, where cGMP was as effective as cAMP (36). Subsequently, two fractions of protein kinase activity could be separated from lobster muscle, which had quite different affinities for cGMP and cAMP. The approximate K_m values of one fraction were 0.08 μM and 4 μM respectively, while

the K_m values of the other fraction were 1.2 μM and 0.02 μM respectively (37).[2] cGMP does not seem to inhibit the formation of cAMP or to alter the effect of cAMP in liver phosphorylase activation.

In intact cell systems, on the other hand, the two cyclic nucleotides produced similar effects when applied in high concentration, that is, under unphysiological conditions, for instance in fat cells (38). In the perfused rat liver, cGMP was ⅓–½ as potent as cAMP in stimulating net glycogenolysis, glucose neogenesis, and release of K^+ (39). Insulin antagonized the effect of exogenous cAMP, but not of exogenous cGMP, on glucose release. The effectiveness of cGMP was explained by its accumulation in liver to a much greater extent than cAMP. In addition, high concentrations of exogenous cGMP can produce an elevation of intracellular cAMP in some tissues, perhaps by inhibiting phosphodiesterase. These results have been partially contested in a recent paper (40). In isolated fat cells incubated in Krebs-Ringer phosphate buffer with Ca^{2+} and Mg^{2+} without K^+, cGMP produced lipolysis but to a much lower degree than cAMP (41). There are contradictory statements concerning the activity of cGMP in stimulating steroidogenesis in intact adrenal cells in vitro (31, 42). The physiological significance in vivo of cGMP seemed therefore doubtful, but more recently some interesting observations were reported. The cGMP levels in the isolated perfused rat heart were elevated by acetylcholine (43). In addition, treatment of mice with oxotremorine increased cGMP in cerebral cortex and in cerebellum (44); later, it has been stated that in mouse cerebellum slices cGMP is not directly involved in cholinergic neurotransmission mechanism (45). These changes both in heart and brain were, however, prevented by the anticholinergic agent atropine. In the presence of high concentration of KCl (125 mM) and of SC-2964, acetylcholine increases the levels of cGMP two- to threefold in some tissues in the presence, but not in the absence, of 1.8 mM Ca^{2+} (46). Db-cGMP as well as carbachol, an acetylcholine analog, decreased the rate of rhythmic beating of culture rat heart cells, while epinephrine and db-cAMP accelerate this rate (47). The conclusion might therefore be drawn that cGMP is involved in some way in cholinergic transmission.

cAMP AND cGMP PHOSPHODIESTERASES

These enzymes, which by hydrolysis of the 3'-O-phosphate bond convert the cyclic nucleotides to 5'-AMP and 5'-GMP respectively, appear as regulatory factors that control the levels of cAMP and cGMP. By Agarose and Sephadex gel filtration, the presence of three fractions could be shown in the brain cortex, the kidney, and the adipose tissues of rats (48, 49). One of these fractions (I) is particulate, the other two (II and III) are soluble and have mol wt of 400,000 and 200,000 respectively, according to gel filtration. The higher mol wt fraction II has for cAMP an apparent K_m of about $1.10^{-4} M$ and for cGMP a K_m of about $1.10^{-5} M$. The hydrolysis of both substrates appears to take place competitively. The lower mol wt fraction III does

[2] cAMP and cGMP-dependent protein kinases have similar components and are inhibited and activated respectively by a protein which is present in lobster tail muscle and has been called "modulator." This factor is similar to the protein inhibitor I (see above) and alters in some cases the substrate specificity of the protein kinases.

not catalyze the hydrolysis of cGMP under normal assay conditions and has for cAMP a K_m of $5.10^{-6}M$ or lower; cGMP is a noncompetitive inhibitor. The particulate fraction I has kinetic properties similar to those of the lower mol wt fraction and is perhaps a membrane bound form of the latter. It was found that the ratio of the high K_m to the low K_m cAMP phosphodiesterases was markedly different in the various areas of the rat brain (50). In rat heart, only fractions similar to II and III could be detected (48). Several authors found in bovine heart two fractions, but their K_m values varied with the methods of separation: e.g. after acrylamide gel electrophoresis and ultracentrifugation, the main fraction (mol wt 125,000 as estimated by analytic ultracentrifugation) had apparent K_m values of 5.10^{-5} and $2.10^{-4}M$ for cGMP and cAMP respectively (51). In rat liver (52) three fractions were separated by DEAE chromatography: two soluble fractions of mol wt 400,000, as determined by gel filtration, and a particulate fraction. One of the soluble fractions hydrolyses cGMP (K_m from 3.10 to $6.10^{-6}M$), and this fraction seems to be a specific cGMP phosphodiesterase whose activity is unaffected by cAMP. The second soluble fraction hydrolyses both cAMP and cGMP (K_m 4.10 and $2.10^{-5}M$ respectively); each substrate acts as a competitive inhibitor of the hydrolysis of the other substrate. cGMP at 1 μM concentration is, however, an activator of cAMP hydrolysis. The particulate fraction has a high affinity for cAMP (K_m $6.10^{-6}M$) and is a cAMP phosphodiesterase, which is inhibited by cGMP in a hyperbolic fashion. More recently, the soluble supernatant fraction of rat cerebellar homogenate was subjected to electrophoresis on a polyacrylamide gel column and six peaks of cAMP phosphodiesterase activity were found (53). The substrate specificities of these peaks are, however, still unknown; the low and high mol wt fractions mentioned above are thus probably mixtures of isoenzymes.

In earlier studies, it was found that the brain cAMP enzyme required Mg^{2+}; Mn^{2+} could completely replace Mg^{2+} while Co^{2+} and Ni^{2+} could only partially replace Mg^{2+}. A soluble fraction of the rat brain enzyme was stimulated by $10^{-6}M$ Ca^{2+} in the presence of $3.10^{-3}M$ Mg^{2+} (54). It has been reported that the activity of cAMP phosphodiesterase from brain was completely dependent on the addition of another brain protein (55).[3] However, this activator stimulated only two of the six peaks obtained by polyacrylamide electrophoresis (53); Ca^{2+} increases the activity of only one of these two peaks.

In summary, phosphodiesterases appear as complex mixtures of different forms hydrolizing cAMP and/or cGMP. Their activity may be regulated by interaction between cAMP and cGMP, and by protein interaction.

Imidazole was reported to stimulate a phosphodiesterase preparation from brain but only at high concentration; this stimulation is therefore not of physiological significance. Methylxanthines (theophylline, caffein) are the classical inhibitors of cAMP phosphodiesterases. Many authors have emphasized the fact that methylxanthines may display numerous pharmacological effects that do not directly involve

[3]Similar protein activators have been found in several mammalian tissues. The bovine heart activator enhances the V_{max} of the cAMP-dependent bovine heart phosphodiesterase and decreases the K_m for cAMP.

phosphodiesterase inhibition. SC-2964 is a more potent xanthine derivative. Several other inhibitors have been discovered: puromycin, triiodothyronine, ATP, diazoxide, chlorpropamide, tolbutamide, and papaverine. Imidazole derivatives may be strong inhibitors; among them, RO-201724 is the most potent compound and has been reported to inhibit selectively the hydrolysis of cAMP as compared to cGMP. SO-20009 is a pyridine derivative. Certain analogs of cAMP are also potent inhibitors.[4] The differences of the chemical structure of all these phosphodiesterase inhibitors is quite remarkable.

SOME ANALOGS OF cAMP

cAMP has in general a weak effect in intact cells, due to the impermeability of cell membranes to phosphorylated compounds and to the destruction by phosphodiesterases. The preparation of synthetic analogs of cAMP started with the idea of obtaining substances endowed with either a better penetration through cell membranes or with a better resistance to the action of phosphodiesterases. The first synthetic analog contained in acyl group, most frequently a butyryl group, in N^6 and/or 2'-O (56, 57). These lipophilic fatty acid residues were introduced in the hope that they might facilitate passage across cell membranes; in a similar way, db-cGMP was prepared. The 2'-OH group has been blocked (56–58), not only by acylation, but also, for instance, by methylation; it is replaced by hydrogen in 2'-deoxy-cAMP. The ribose moiety in cAMP has been replaced by *D*-arabinose or *D*-xylose. The phosphodiester group has been modified by replacement of one of the two oxidic O atoms by S (cyclic phosphorothiotate) or by a CH_2 group. 5'-amido analogs were obtained by replacing 5'-O with NH or NCH_3 (59). Substituted amides (cyclic phosphoramidates) (60) and the methyl ester of cAMP were also prepared (56). Another type of analog contains the ribose moiety attached to the 3-position instead of the 9-position of adenine (cyclic iso-AMP) (61). A series of analogs were prepared that involved modifications of the adenine skeleton: cAMP N^1-oxide (56, 57); C–2, C–6 (62–64), and C–8 (65, 66) substituted compounds. The C–6 NH_2 group and the C–8 hydrogen have thus been replaced by halogens or by NHR, NR_1R_2, OH, OR, SH, and SR groups. Finally, in tubercidine-3',5'-phosphate N–7 is replaced by a CH group (67).

The effects of some of these analogs have been investigated on enzyme preparations. The unblocked 2'-OH in the ribo-configuration was found to be necessary for the activation of cAMP dependent protein kinases (58). 2'-O-substituted analogs were attacked somewhat more slowly than cAMP by several phosphodiesterases, and they inhibit moderately the action of these enzymes on cAMP, this effect being dependent on the nature of the substituent and on the origin of the enzymes. Among the 8-substituted analogs, the 8-thio, the 8-hydroxy, and the 8-amino derivatives were more active in decreasing order than cAMP towards bovine, brain, or liver protein kinases (58, 68). They were relatively resistant to rabbit kidney and rat brain phosphodiesterases and were good inhibitors of these enzymes, the 8-thio-derivative being especially active (58, 67). Db-8-thio-cAMP and 8-thio-cAMP at fivefold

[4]Other phosphodiesterase inhibitors occur in nature, e.g. a protein factor in amoebae and cytokinesins in higher plants.

higher concentration than cAMP produced a 80% and 30% inhibition respectively of the action of rat brain phosphodiesterase; on the other hand, at much lower concentration, both derivatives produced a 30% activation (69). The 6-substituted analogs were more readily cleaved than the 8-substituted ones by pig and rat brain phosphodiesterase, with the exception of N^6-mb-cAMP, which is quite resistant. Some 6-alkylthio-derivatives were more effective than cAMP as activators of cAMP-dependent bovine brain protein kinases. The 6-thio-derivative was 70% more active than cGMP towards the cGMP-dependent lobster kinase (64). Tubercidine-3',5'-phosphate was as effective as cAMP as an activator of dog heart and liver phosphorylase kinase kinase (67).

The study of the effects on intact cells gave the following results. While cAMP was practically inactive, db-cAMP, N^6-mb-cAMP, and 2'-O-mb-cAMP activated the phosphorylase in rat (68) and dog (70) liver slices and produced hyperglycemia in the intact dog (56); cAMP, db-cAMP, and several 8-substituted derivatives were examined for their ability to activate steroidogenesis in the isolated rat adrenal cells and lipolysis in the rat epididymal lipocytes (71). The maximal activation of most of these compounds was similar, but the concentrations required to produce half maximal activities were different; according to this criterion, the 8-methylthio, the 8-bromo, the 8-hydroxy cAMP, dbc-cAMP, and cAMP displayed activities in decreasing order. Extensive investigations were carried out on the release in vitro of hormones from rat anterior pituitaries. N^6-alkyl derivatives (with the exception of N^6-*t*-butyl cAMP where the substituent is too bulky) and iso-cAMP were more effective than cAMP in increasing TSH release (62). The same derivatives and also 8-bromo-cAMP and 8-thio-cAMP increased more effectively than cAMP the release of GH, but not the release of prolactin. The effect of 8-thio-cAMP was considerably enhanced by dibutyrylation (65). Iso-cAMP, which is quite active on TSH release, was far less potent on GH release (72). It has been found that cAMP and the potent analogs, which produce in vitro significant release of GH from pituitaries, are more active on male rats than on female or castrated male rats. On the other hand, 3–6 $\times 10^{-3}$ cGMP produced an essentially opposite pattern (69).

Db-cAMP is now a very popular compound, which has been used in a multitude of biological experiments that cannot be reported here. Its action on intact cells is generally better than the action of cAMP, although a number of exceptions have been reported. According to a recent publication (73), db-cAMP is taken up by HeLa cells to a much higher degree than cAMP; by the action of an esterase, it is converted to N^6-mb-cAMP, which accumulates inside the cells. This monobutyrate is bound by the R unit of kinases as well as cAMP itself and represents perhaps the active form. Other authors working with thyroid cells (74) found a considerable uptake of exogenous cAMP and they suggested that the high levels of intracellular cAMP may generate the production of a cAMP inhibitor (75) which has been detected in a variety of tissues. The formation of this inhibitor was reported to be prevented by N^6-mb-cAMP.

The mechanism of action of biologically active analogs that do not contain acyl groups involves perhaps again a better penetration through cell membranes; their resistance to phosphoesterases and, in some cases, their inhibitory effects on these enzymes, may also play a role, as in the case of the acyl derivatives.

cAMP AND GENE TRANSCRIPTION OR TRANSLATION

cAMP stimulates the synthesis and induction of a number of enzymes. The role of a cAMP dependent histone kinase has been suggested in higher organisms (76). A stimulation of DNA synthesis occurs in the parotid gland in response to isoproterenol, a potent β-agonist that enhances the level of cAMP (77). At the molecular level, the most penetrating investigations are concerned with the synthesis of inducible enzymes in bacteria, especially in *Escherichia coli*. The presence of cAMP in this microorganism was detected a few years ago; its concentration inside the cells was lowered by glucose (78); in addition, the formation of enzymes induced by a new substrate is repressed by glucose. It was then discovered that exogenous cAMP overcame this repression of the synthesis of β-galactosidase and of other inducible enzymes (79). At the level of DNA, the lac operon, which effects the synthesis of β-galactosidase, is composed in sequence of three regulatory genes (i, p, and o) and of three structural genes (z, y, and a). The latter code for β-galactosidase, galactoside permease, and thiogalactoside transacetylase respectively. The i gene codes for a repressor, which binds to the operator gene o, thereby preventing the transcription, that is the synthesis of mRNA. The new substrate (inducer), for instance isopropyl-β-thiogalactoside (IPTG), stimulates lac mRNA synthesis by binding to the repressor and reduces its affinity for the o gene. The p or promotor gene controls the maximum rate of the lac operon expression. Mutants of *E. coli* were isolated that were unable to metabolize various substrates even in the presence of cAMP. These mutants were subsequently found to lack a protein that could bind cAMP and that has been named the cAMP receptor protein (CRP) (80) or the catabolic gene activator protein (CAP) (81). Purified CRP has a mol wt of 45,000; it is composed of two identical subunits and is very basic; it has no detectable protein kinase activity (82). When lac DNA, RNA polymerase, CRP, and cAMP are incubated together in vitro in the absence of the nucleoside triphosphates, which are the substrates of RNA polymerase, a rifamycin resistant complex is formed. As rifamycin rapidly inactivates free RNA polymerase, the latter is contained in the complex in a protected form. Upon addition of nucleoside triphosphates, lac mRNA is formed (83). It seems likely that RNA polymerase binds at the promotor, for when DNA containing a defective promotor is used in vitro, no RNA is made. A hypothetic model involves first the formation of the complex cAMP-CPR which, due to an allosteric change of CPR, binds to the promotor site close to the lac operator. A conformational modification of DNA takes place so that RNA polymerase can bind to p. In the presence of nucleoside triphosphates, the transcription ensues. The formation of gal mRNA is supposed to take place in a similar way; this mRNA is involved in the synthesis of one of the enzymes of galactose metabolism. These models do not involve any phosphorylation.

The effect of cAMP seems thus to occur at the transcriptional level. Another mechanism is, however, probably involved in the synthesis of another inducible enzyme, tryptophanase. cAMP stimulates this synthesis when it is added after mRNA synthesis has been arrested either by the removal of the inducer (tryptophane) or by treatment with actinomycin D or proflavin. This suggests that cAMP

was acting in this case at the translational level to increase the peptide chain elongation (84).

A possible mechanism of the action of cAMP at the translational level is suggested by the following work. cAMP is bound in the presence of GTP and Mg^{2+} to a second protein, the G translocation factor, in addition to the CPR factor required for β-galactosidase synthesis. The G factor functions as or with a ribosome-dependent GTPase in the process by which the tRNA molecules are shifted on the ribosome as it moves along mRNA (85). cAMP would thus be involved in the GTP conversion to GDP, which releases the energy required for translocation. Another example of action at the translational level is afforded by a work on liver tyrosine transaminase, where cAMP releases the enzyme from polysomes bound to membranes; in addition, a soluble factor is required (86).

CELL PROLIFERATION

Contradictory reports can be found concerning the effect of cAMP on cell mitosis, especially in cancerous tissues. High levels of cAMP have been considered as a stimulating factor, e.g. in parotid gland (77, 87); however, other investigations point to a defect in cAMP formation as a concomitant of abnormal cell proliferation. The exogenous cAMP was reported to inhibit the growth of two virus-transformed derivatives of a line of hamster kidney fibroblast, in tissue culture (88). Cells transformed by a polyoma virus contained lower adenyl cyclase activity than control cells or cells transformed by Rous sarcoma virus (89). The growth of HeLa cells and of a line of chick fibroblast was inhibited by exogenous cAMP (90). These tissue culture studies were extended with similar results to other cell lines. It was reported that Rous sarcoma virus-induced hamster tumor cells, when treated with cAMP or with db-cAMP, regain some morphological and growth characteristics of normal fibroblasts (91). Db-cAMP slows growth and decreases saturation density of cell lines like 3T3, which present contact inhibition. In the skin disease called psoriasis, a four- to twelvefold accelerated epidermal cell division occurs as well as an accumulation of glycogen. cAMP concentrations and adenyl cyclase contents are lower in psoriatic skin than in normal skin; in addition, db-cAMP inhibited epidermal cell division (92–94). It has even been reported that derivatives of cAMP could suppress the growth of tumors in experimental animals (95, 96).

Lymphocytes are cells whose proliferation is stimulated by antigenes. Two mitogenic agents, phytohemagglutinin and concanavaline A at optimal concentration produce a ten- to fiftyfold increase in the concentration of lymphocyte cGMP within the first 20 min of exposure. On the other hand, no change was seen in the concentration of cAMP (97).

The conclusion has been drawn that an increase of the level of cellular cGMP may represent one of the active signals that induce cell division, while the elevation of the cAMP level may limit or inhibit mitosis. Many other experiments will be necessary in order to confirm these considerations, which are still partially hypothetic.

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