Inhibition of Marrow Growth by Cyclic AMP[1] (35831)

ALEC MORLEY, PETER QUESENBERRY, MARIANNE GARRITY, AND FREDERICK STOHLMAN, JR.

Department of Medicine and Research, St. Elizabeth's Hospital, Tufts University School of Medicine, Boston, Massachusetts 02135

Heldrick and Ryan (1) recently reported that low concentrations of cyclic 3, 5-adenosine monophosphate (cAMP) inhibit the growth of one nonmalignant and several malignant cell lines and suggested that this substance might act as a regulator of cell growth. This suggestion is an attractive one since cAMP has been implicated as an intermediary in the action of a variety of hormones including epinephrine (2), and epinephrine and several of its analogues (3–6) have been reported to influence various proliferative characteristics of a number of tissues. Therefore, we used normal mouse bone marrow cells cultured in soft agar to study the effect on cell proliferation of cAMP and of various substances believed to influence the production or destruction of cAMP.

Materials and Methods. Bone marrow cells were obtained from the tibias of 12–16-week-old CF_1 mice (Carworth Farms) and were cultured by the technique of Bradley and Metcalf (7) as modified by Rickard *et al.* (8). For each measurement, 3–5 plates were set up, each containing 1×10^5 cells suspended in 1 ml of agar medium. Serum from irradiated or endotoxin-treated mice was used as a stimulatory agent and 0.05 ml was added to each plate. Colonies were counted after incubation of the plates for 7–8 days at 37° in a humidified atmosphere of 7.5% CO_2 in air. The following chemicals were tested for their effect: cAMP (Sigma), dibutyryl cAMP (Sigma), epinephrine (Parke-Davis), aminophylline (Searle), Imidazole (Sigma), adenosine (Sigma), adenosine monophosphate (AMP, Sigma), adenosine diphosphate (ADP, Sigma), adenosine triphosphate (ATP, Sigma), insulin (Lilly) and nicotinic acid (Lilly). Where necessary, the substance was dissolved in modified Eagles medium, sterilized by passage through a 0.45-μ Millipose filter, and various dilutions were then made. When a stimulatory effect was being sought, 0.1-ml aliquots were added to each otherwise unstimulated culture; when inhibition was being measured, either 0.05 ml was added to each stimulated culture if only one substance was being tested or 0.025 ml of each solution was added if 2 substances were being tested simultaneously.

Results. An inhibitory effect on colony formation was produced by cAMP in each of 4 experiments, by dibutyryl cAMP in each of 2 and by epinephrine and aminophylline in each of 3. For any one substance, the results of replicate experiments were quantitatively similar and results from representative experiments for each substance are shown in Fig. 1. On 2 occasions the inhibitory properties of dibutyryl derivative had a greater inhibitory effect than the parent compound. The inhibitory effect of cAMP could not be reversed by simultaneous addition of imidazole (final concentration of 1.2×10^{-3} *M*), insulin (2 units/ml) or nicotinic acid (2.5×10^{-4} *M*). The effect of various adenine derivatives in stimulated cultures was studied in one experiment and the results are given in Table I; all showed some degree of inhibition of stimulated cultures. When these compounds were added to unstimulated cultures, no effect was seen.

Discussion. In the present study, addition of cAMP to cultures of normal mouse marrow cells inhibited the ability of these cells to form granulocytic colonies *in vitro*. Addition

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TABLE I. Inhibition of Marrow Growth Produced by the Addition of Various Adenine Derivatives in Different Concentrations.
The results are the mean number of colonies ± 1 standard error.

Control	Additive	Conc/plate		
		0.75 m*M*	0.075 m*M*	0.0075 m*M*
56 ± 3	cAMP	38 ± 2	43 ± 6	53 ± 8
	Dibutyryl cAMP	1 ± 1	2 ± 1	22 ± 4
	Adenosine	22 ± 4	36 ± 3	46 ± 1
	AMP	4 ± 1	14 ± 1	50 ± 3
	ADP	18 ± 2	39 ± 7	51 ± 2
	ATP	18 ± 3	36 ± 3	34 ± 2

of the dibutyryl derivative of cAMP, which is thought to result in a higher intracellular level of cAMP (2), produced even greater inhibition. Addition of epinephrine and aminophylline, which raise the intracellular level of cAMP by influencing the balance between production and destruction (2), also resulted in inhibition. Since erythroid colonies do not develop in the culture system used in these experiments, the decreased number of colonies observed may reflect true inhibition of colony growth or may indicate that stem cells were induced to differentiate along the erythroid pathway and thus lost their ability to form granulocytic colonies *in vitro*. This latter possibility would be in accord with a recent report that dibutyryl cAMP increased the *in vitro* incorporation of iron into heme. (9). In either case the results could be interpreted as indicating that cAMP is a determinant of marrow cell proliferation and differentiation, with the implication that humoral regulation of blood cell production might be mediated through control of the intracellular level of cAMP. However the remainder of our results, while not eliminating this possibility, failed to provide further support for it. Addition of insulin, nicotinic acid, and imidazole, all substances which are believed to decrease intracellular levels of cAMP (2), did not result in a decrease in the inhibitory effect of simultaneously added cAMP. Furthermore a number of adenine derivatives other than cAMP also had an inhibitory effect, which raises the possibility that the observed effect of cAMP was nonspecific. In

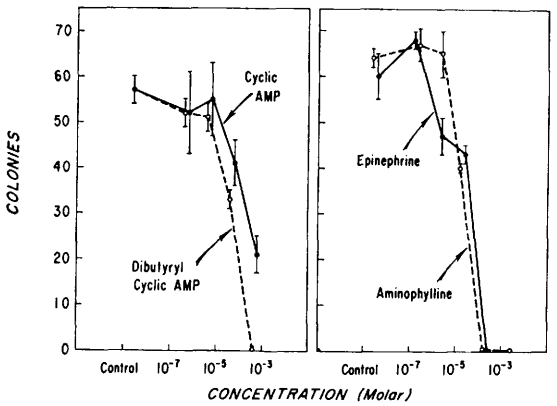

FIG. 1. Inhibition of marrow growth produced by cAMP, dibutyryl cAMP, epinephrine, and aminophylline: Each point shows the mean number of colonies ± 1 standard error. The concentration refers to the final concentration per plate of the various additives.

a recent study of a different system, lymphocyte transformation induced by phytohemagglutinin, Hirschorn *et al.* (10) and Smith *et al.* (11) observed results very similar to our own; cAMP, dibutyryl cAMP, and aminophylline inhibited cell proliferation but various adenine nucleotides did likewise. Both of these groups of workers concluded from these results that the case for cAMP being a physiological regulator of cell growth was not proven and, with respect to the present study, a similar conclusion would seem justified.

Summary. Cyclic AMP, dibutyryl cyclic AMP, epinephrine, and theophylline inhibited growth of marrow colonies *in vitro*. The interpretation of this observation is uncertain since various adenine nucleotides had a similar effect and since several substances lowering intracellular cyclic AMP levels were unable to reverse the inhibitory effect of exogenous cyclic AMP.

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