The content and metabolism of cyclic adenosine 3', 5'-monophosphate and cyclic guanosine 3', 5'-monophosphate in adenocarcinoma of the human colon.

F R DeRubertis, … , R Chayoth, J B Field

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Research Article

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The Content and Metabolism of Cyclic Adenosine 3′, 5′-Monophosphate and Cyclic Guanosine 3′, 5′-Monophosphate in Adenocarcinoma of the Human Colon

FREDERICK R. DERUBERTIS, REUBEN CHAYOTH, and JAMES B. FIELD

From the Department of Medicine, Veterans Administration Hospital, and University of Pittsburgh and the Clinical Research Unit of Presbyterian-University Hospital, Pittsburgh, Pennsylvania 15240

ABSTRACT Data from cultured cells have suggested that cyclic AMP and cyclic GMP may be important determinants of cell growth and transformation. However, few studies have examined cyclic nucleotide content and metabolism in naturally occurring tumors of man. Accordingly, in the present study we compared cAMP and cGMP levels and metabolism in carcinomas of the human colon to those of the adjacent uninvolved mucosa after therapeutic resection of these tissues. The cAMP content of the tumors, determined in samples frozen 30 min after excision, was significantly lower than that of the adjacent mucosa, when expressed on the basis of tissue wet weight, protein, or DNA content. By contrast, the cGMP content of the tumors was higher than that of the surrounding mucosa if calculated on the basis of tissue wet weight, but this difference did not persist when correction was made for the higher protein or DNA content of the tumors. Incubation of slices of mucosa or tumor with or without theophylline in vitro increased tissue cAMP and cGMP content above levels observed in frozen samples of the same tissue. However, after such incubations cAMP levels in the tumors remained clearly below that of the mucosa, while cGMP content of the two tissues did not differ. The failure of theophylline to abolish differences in cAMP content and the comparable activities of high and low K_m cAMP-phosphodiesterase in homogenates of the two tissues suggested that the lower cAMP content of the tumors was a consequence of diminished cAMP synthesis rather than enhanced degradation. This possibility was supported by the reduction in basal and maximal prostaglandin E_1 (PGE_1)-responsive adenylate cyclase activity found in tumor homogenates relative to those of mucosa, and the lower levels of cAMP in tumor slices after incubation of the tissues with a maximal dose of PGE_1 and theophylline. Since NaF-responsive adenylate cyclase activity was not significantly reduced in the tumors, the lower basal and PGE_1 activities may not be related to a deficiency of the catalytic unit of the cyclase complex in this tissue. The role of reduced activity of the adenylate cyclase-cAMP system and/or reduced tissue cAMP-to-cGMP ratios in the pathogenesis of colonic carcinoma is uncertain, but these changes might favor unregulated cellular proliferation.

INTRODUCTION

There is considerable evidence that cyclic adenosine 3′,5′-monophosphate (cAMP) [1] and cyclic guanosine 3′,5′-monophosphate (cGMP) are important determinants of cell growth and differentiation (1–13). The precise role of each of these nucleotides in the control of cell proliferation is unresolved. However, cAMP inhibits growth of several cell lines in vitro (10), and restores contact inhibition and normal morphologic features to noncontact-inhibited, transformed fibroblasts (2, 3, 14, 15). By contrast, cGMP stimulates nucleic acid synthesis and

Dr. DeRubertis is a Clinical Investigator of the VA Hospital and recipient of the American College of Physicians Teaching and Research Scholarship.

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[1] *Abbreviations used in this paper:* Ca, carcinoma; cAMP, cyclic adenosine 3′,5′-monophosphate; cGMP, cyclic guanosine 3′,5′-monophosphate; KRBG, Krebs-Ringer bicarbonate buffer containing glucose and bovine serum albumin; Muc, mucosa; TCA, trichloroacetic acid.

growth of cells in culture (10, 12, 13). These observations have led to the hypothesis that cAMP and cGMP may be biologic antagonists in the regulation of cell growth (16, 17). According to this concept, derived primarily from studies of cultured cells, a reduction in cAMP, an increase in cGMP, or both might be predicted in tissue that has undergone malignant transformation. Determination of cyclic nucleotide levels in experimentally induced tumors, particularly rodent hepatomas, has not revealed a consistent pattern of alteration in cyclic nucleotide content relative to that of the surrounding normal tissue (18–21). However, only limited data are available on cyclic nucleotide levels or the regulation of cyclic nucleotide metabolism in naturally occurring neoplasms of man (22, 23). Accordingly, in the present study, we examined the cAMP and cGMP levels and cyclic nucleotide metabolism in carcinoma of the human colon. This tumor was chosen for study because it occurs commonly, and a portion of adjacent uninvolved mucosa, routinely resected with the tumor, is also available for comparative biochemical analysis.

METHODS

Preparation of tissue. After segmental colonic resections, specimens were immediately placed in 0.85% saline at 0–4°C, and portions of the tumor and the adjacent uninvolved colon were obtained for biochemical studies. Uninvolved colonic mucosa was manually separated from the submucosa, and samples of the mucosa and segments of the tumors were then frozen in liquid nitrogen within 30 min of resection for subsequent determination of cAMP, cGMP, protein, DNA content, and activities of cAMP-phosphodiesterase, as described below. A portion of each tissue (100 mg/ml buffer) was minced and gently homogenized in 10 mM Tris buffer (pH 7.4) for assay of adenylate cyclase activity on the day of resection. In several experiments, slices of each tissue were also prepared within 30–40 min of excision, with a Stadie-Riggs microtome. Groups of triplicate slices were incubated at 37°C in 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 1 mg/ml of glucose and bovine serum albumin (KRBG), with or without 10 mM theophylline. After incubation of slices under the conditions indicated in the Results, tissue was homogenized in 5% trichloroacetic acid (TCA) at 4°C and cyclic nucleotide content extracted and assayed as described below.

Uninvolved colonic mucosa was also obtained for study from patients undergoing segmental colonic resections for diverticular disease. All tissue specimens or extracts were stored at −70°C and assayed within 1 wk.

Extraction and assay of cAMP and cGMP. Approximately 150 mg of frozen tissue was homogenized at 4°C in 1.5 ml of 5% TCA containing 2,000 cpm of [^{3}H]cAMP (sp act, 38 Ci/mmol, New England Nuclear, Boston, Mass.). Homogenates were centrifuged at 4,000 rpm for 15 min at 4°C and supernate extracted three times with 15 ml of ethyl ether saturated with water. Tissue slices (approximately 50 mg) were similarly homogenized in 0.5 ml of 5% TCA, at the termination of the timed incubations, and the supernates stored for subsequent extraction with ether (5 ml × 3). Extracts were dried with a Rotary Evapo-Mix (Buchler Instruments Div., Searle Analytic Inc., Fort Lee, N. J.) at 60°C. Residues were resuspended in 800 μl of water. For assay of cGMP, 400 μl of this solution was adjusted to pH 6.2 with 100 μl of 250 mM sodium acetate buffer. For assay of cAMP, 200 μl of the solution was adjusted to pH 4.0 with 50 μl of 250 mM sodium acetate buffer. Routinely, cyclic nucleotide concentrations of these solutions were determined in duplicate (100 and 200 μl in the cGMP assay; 25 and 50 μl in the cAMP assay). Recoveries of both nucleotides were estimated from the recovery of added [^{3}H]cAMP, since in preliminary studies recoveries of [^{3}H]cAMP and [^{3}H]cGMP (sp act 16 Ci/mmol, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) did not differ significantly in duplicate extractions of the same tissues. Recoveries ranged from 70 to 80%.

cAMP was assayed by the protein-binding method in 50 mM sodium acetate buffer (pH 4.0), as previously described (24).

cGMP was determined by the radioimmunoassay procedure of Steiner et al. (25). Anti-cGMP antibody and [^{125}I]-succinyl cGMP tyrosine methyl ester (sp act 600–800 mCi/mg) were obtained commercially (Schwarz/Mann). Labeled antigen, antibody, standards, or unknowns were incubated in 1 ml of 50 mM sodium acetate buffer (pH 6.2) for 18 h at 4°C. Blanks contained labeled antigen without antibody. After incubation, antibody-antigen complexes were precipitated and washed at 4°C with 2.5 ml of 60% saturated ammonium sulfate. Samples were counted in a Packard Autogamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). In the absence of added cold cGMP, approximately 50% of labeled antigen was bound to antibody. Standard curves were plotted as logit-log functions, with the percentage of total labeled antigen bound as the

TABLE I

cAMP Content of Uninvolved Mucosa and Adenocarcinomas of the Colon

Patient	Muc	Ca	Muc	Ca	Muc	Ca
	pmol/g wt		*nmol/g protein*		*pmol/mg DNA*	
1	980	370	10.4	4.1	280	128
2	760	400	6.4	4.1	162	89
3	650	510	8.8	4.4	159	80
4	1,030	690	11.2	7.9	322	182
5	740	230	9.1	2.6	239	85
6	580	350	8.6	4.7	166	83
7	562	416	6.6	4.1	126	80
8	762	751	9.4	9.0	202	242
9	428	397	6.3	4.7	89	53
10	453	320	3.8	2.3	104	71
11	361	328	3.1	1.7	77	31
12	549	392	4.3	2.4	86	27
13	893	715	8.7	4.5	198	159
Mean	670	451*	7.5	4.3*	170	101*
SE	58	45	0.7	0.6	21	17

cAMP content of tissue specimens, frozen approximately 30 min after therapeutic surgical resection.

* $P < 0.005$ comparing carcinomas (Ca) to uninvolved mucosa (Muc) by paired analysis.

TABLE II

Protein and DNA Content of Uninvolved Mucosa and Adenocarcinomas of the Colon

Patient	Protein		DNA	
	Muc	Ca	Muc	Ca
	mg/g wet wt			
1	94	91	3.5	2.9
2	115	97	4.7	4.5
3	73	116	4.1	6.4
4	92	88	3.2	3.8
5	81	89	3.1	2.7
6	67	75	3.5	4.2
7	85	101	4.6	5.2
8	77	84	3.6	3.1
9	68	85	4.8	7.5
10	120	138	4.4	4.5
11	114	143	4.7	7.8
12	126	163	4.2	9.1
13	103	160	4.5	4.5
Mean	94	110*	4.1	5.1
SE	6	9	0.2	0.6

* $P < 0.025$ comparing value in tumor (Ca) to adjacent mucosa (Muc) by paired analysis.

logit and the amount of "cold" cGMP added (0.1–10 pmol) as the log function. Data was analyzed by computer (University of Pittsburgh Computer Center, Pittsburgh, Pa.), by methods of calculation previously reported (26). The lower limits of assay sensitivity was 0.1 pmol cGMP. cAMP (5 nmol), guanosine (1 nmol), 5'-GMP (1 nmol), and GTP (1 nmol) did not cross-react with the antibody. Validity of cAMP and cGMP measurements was also verified by the linearity of sample dilutions, cyclic nucleotide destruction with phosphodiesterase, and quantitative recovery of "cold" cyclic nucleotides added to TCA homogenates of tissues (data not shown).

Adenylate cyclase activity was examined at 30°C in homogenates of tissue, by the conversion of [α-^{32}P]ATP into [^{32}P]cAMP, according to methods previously described (22). Formation of cAMP was linear for the 5-min reaction period. Phosphodiesterase activities of homogenates were examined at 30°C by the method of Thompson and Appleman (27), as previously described (28). Activity was examined at a high (0.1 mM) and low (0.13 μM) cAMP concentration. Dilutions of tissue homogenates were employed that destroyed approximately 20% of added cAMP. Under these conditions, hydrolysis of cAMP was linear for the 5-min reaction period. Protein was determined by the Lowry et al. method (29) and DNA by the method of Burton (30).

Significance of statistical differences between mean values were examined by Student's t test for paired values. Limitation in the quantity of tissue available for biochemical study prevented assessment of all of the above parameters in every tissue specimen.

RESULTS

The tumors studied were all classified histologically as adenocarcinomas. These originated predominantly in the rectosigmoid and descending colon, although three cecal carcinomas are included in this series (patients 3, 7, and 12). Tumors varied in their degree of morphologic differentiation, in their hypercellularity relative to that of the surrounding mucosa, and in their content of mucin or fibrous tissue. Because of this histologic heterogeneity, cyclic nucleotide content and enzyme activities were expressed on the basis of tissue wet weight, protein, and DNA content.

cAMP and cGMP content. Table I shows cAMP levels in uninvolved mucosa (Muc) and carcinomas (Ca) from 13 patients, expressed on the basis of tissue wet weight, protein, and DNA content. In every patient but 8, cAMP levels in the tumors were reduced relative to those of the adjacent mucosa by each method of calculation. Protein content of Ca was significantly higher than that of Muc. DNA content of Ca was somewhat higher than that of Muc but this difference was not of statistical significance (Table II). As shown in Table III, mean cGMP content of Ca was higher than that of Muc when expressed on the basis of tissue wet weight, but not when analyzed on the basis of protein or DNA content. However, when compared to the value for Muc from the same patient, the ratio of tissue cAMP to cGMP content was significantly decreased in Ca (Table IV). This decrease was primarily a consequence of the lower levels of cAMP in Ca, but the higher content of cGMP found in some tumors also contributed to the

TABLE III

cGMP Content of Uninvolved Mucosa and Adenocarcinomas of the Colon

Patient	Muc	Ca	Muc	Ca	Muc	Ca
	pmol/g wet wt		*pmol/g protein*		*pmol/mg DNA*	
1	75	85	795	934	21	29
2	41	44	357	453	9	10
3	39	49	530	426	10	8
4	36	38	392	445	11	10
5	62	56	763	627	20	21
6	46	51	685	678	13	12
7	29	72	342	713	6	14
8	32	40	416	480	6	13
9	21	67	309	797	5	9
10	60	47	498	338	14	10
11	41	69	357	483	9	9
12	47	66	371	421	11	8
13	41	58	398	365	9	13
Mean	44	57*	478	551	11	13
SE	4	4	46	51	1.4	1.7

cGMP content of tissue specimens, frozen approximately 30 min after surgical resection.

* $P < 0.025$ comparing carcinomas (Ca) to uninvolved mucosa (Muc) by paired analysis.

TABLE IV

cAMP/cGMP Ratios in Uninvolved Mucosa and Adenocarcinomas of the Colon

Patient	Muc	Ca
1	13.3	4.4
2	18.0	8.9
3	15.9	10.0
4	29.3	18.2
5	12.0	4.1
6	12.8	6.9
7	21.0	5.7
8	33.7	18.6
9	17.8	5.9
10	7.4	7.1
11	8.6	3.4
12	7.8	3.4
13	22.0	12.3
Mean	16.9	8.4*
SE	2.2	1.4

Ratios of tissue cAMP to cGMP content, calculated from cyclic nucleotide levels shown in Tables I and III.
* $P < 0.005$, comparing carcinoma (Ca) to adjacent mucosa (Muc).

change. Among the various patients studied, there was considerable overlap in the absolute values of cAMP, cGMP, and in the cAMP/cGMP ratios of Muc compared to Ca (Tables I, III, and IV). Similarly, the mean values for cAMP (758±SE 62 pmol/g wet wt) and cGMP (47±5) in uninvolved colonic mucosa obtained from five patients treated by segmental colonic resection for diverticular disease were not distinguishable from

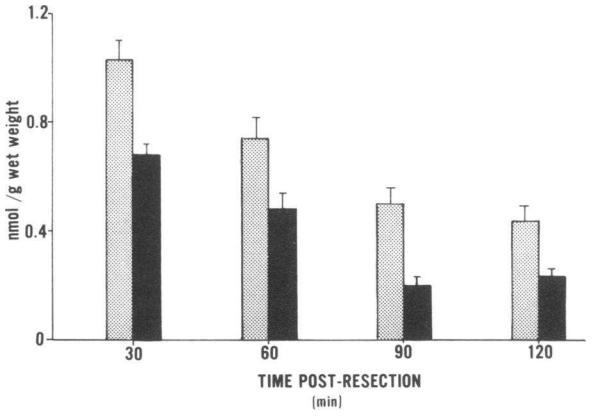

FIGURE 1 Alterations in cAMP content of colonic carcinomas (solid bars) and adjacent mucosa (stippled bars) as a function of time after resection. Tissue was maintained at 4°C in saline and triplicate samples of tumor and mucosa were quick-frozen at the times shown. Values are mean±SE from a single experiment.

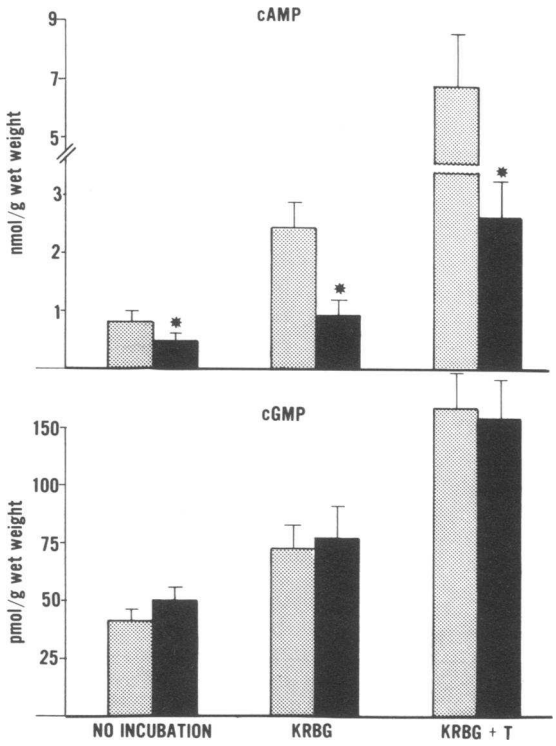

FIGURE 2 Effects of in vitro incubation on the cAMP and cGMP content of carcinomas (solid bars) and adjacent uninvolved mucosa (stippled bars). Groups of triplicate slices of tissues from five patients were incubated at 37°C for 20 min in Krebs Ringers bicarbonate glucose buffer in the absence (KRBG) or presence of 10 mM theophylline (KRBG + T). These values (mean±SE) were compared to cyclic nucleotide levels in tissue frozen 30 min after resection without in vitro incubation. * $P < 0.025$ comparing carcinoma and adjacent mucosa for the same condition.

levels observed in the mucosa adjacent to carcinomas, and overlap in the nucleotide content of mucosa obtained from each population was evident.

Since tissue samples could not be quick-frozen *in situ*, the effect of delays in tissue processing on the relative content of cyclic nucleotides in Muc and Ca was examined. As shown in Fig. 1, cAMP levels in both tissues declined progressively when specimens were maintained at 0–4°C in saline for a 120-min period. However, differences between the two tissues were detectable at each time point examined. Similarly, cGMP levels declined with time when tissue processing was delayed, but relative differences between Muc and Ca were maintained (not shown). Fig. 2 shows the alteration in tissue cAMP and cGMP content occurring during 20-min incubations of slices of Muc and Ca in the presence or absence of 10 mM theophylline. In comparison to levels in unincubated tissue frozen 30 min after surgical re-

section, in vitro incubation significantly increased levels of cAMP and cGMP in both Muc and Ca. Higher values were observed in slices incubated with theophylline. However, under each condition, cAMP content of Ca remained significantly below that of Muc. By contrast, cGMP content of the two tissues did not differ after a 20-min incubation with or without theophylline. The cAMP responsiveness of the two tissues to PGE_1 is shown in Fig. 3. In the presence of theophylline, a maximal dose of PGE_1 (20 μM) significantly enhanced the cAMP content of both Muc and Ca (approximately three-fold over basal), but absolute levels of cAMP in Muc after incubation with PGE_1 were considerably higher than those of Ca. From the above results it seemed unlikely that the lower cAMP observed in frozen specimens of Ca was a simple consequence of accelerated nucleotide turnover in this tissue between the time of resection and freezing.

Fig. 4 shows the basal adenylate cyclase activities of homogenates of Ca and Muc. When values were expressed on the basis of tissue wet weight, differences between the two tissues were not statistically significant, although mean values were lower in Ca. When corrected for tissue protein or DNA content, enzyme activity was

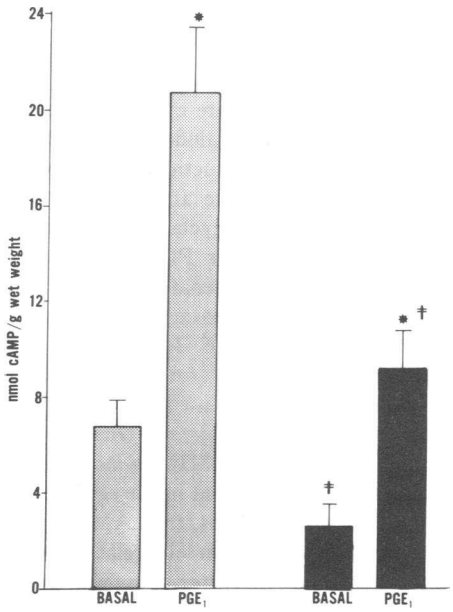

FIGURE 3 Effects of prostaglandin E_1 (PGE_1) in vitro on the cAMP content of carcinomas (solid bars) and adjacent uninvolved mucosa (stippled bars). Groups of triplicate slices of each tissue were incubated for 20 min in buffer with 10 mM theophylline in the presence or absence of a maximal dose of PGE_1 (20 μM). Values represent mean ±SE from eight tumors and adjacent mucosa. * $P < 0.001$ comparing PGE_1 and basal of the same tissue, ‡ $P < 0.005$ comparing basal or PGE_1 of the tumors to that of the mucosa.

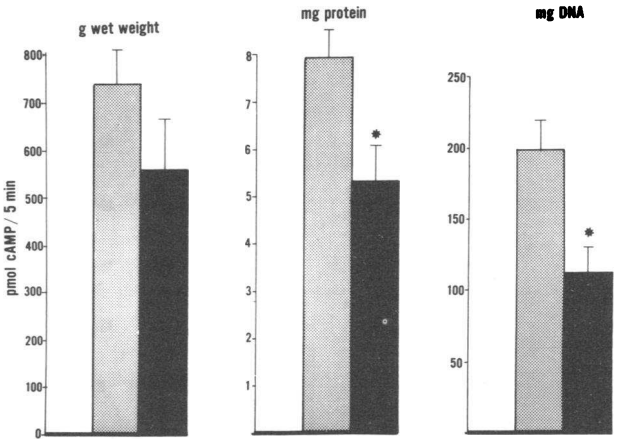

FIGURE 4 Basal adenylate cyclase activities of 13 carcinomas (solid bars) and adjacent mucosa (stippled bars), expressed as pmol cAMP formed/5 min per g tissue wet wt, and per mg protein or DNA. Enzyme activity was assayed at 30°C in whole tissue homogenates, with a reaction time of 5 min. Values represent mean±SE of triplicate determinations from 13 experiments. * $P < 0.025$ comparing tumors to mucosa.

significantly reduced in Ca. As shown in Fig. 5, maximal PGE_1-responsive adenylate cyclase activity of Ca, assessed in six tumors, was also lower than that of Muc when expressed on the basis of tissue protein or DNA content but not on the basis of wet weight. By contrast, NaF-responsive adenylate cyclase activity of Ca did not differ significantly from that of Muc by any method of calculation.

cAMP-phosphodiesterase activity was examined in eight tumors and the surrounding mucosa at both a low (0.13 μM) and high (0.1 mM) substrate concentration. Whether expressed on the basis of tissue weight wet, protein, or DNA, hydrolysis of cAMP by the tumors at each substrate concentration did not differ significantly from that of the adjacent mucosa. However, the high K_m enzyme activity was somewhat lower in Ca (mean 2,217±SE 209 pmol cAMP hydrolyzed/5 min/mg protein) than in Muc (2,630±224).

DISCUSSION

The results of the present study demonstrate that the cAMP content of adenocarcinomas from human colon is lower than that of the adjacent uninvolved mucosa. This reduction was evident whether cAMP content was expressed on the basis of tissue wet weight, protein, or DNA content. By contrast, cGMP content of the tumors was greater than that of the surrounding mucosa if calculated on the basis of tissue wet weight, but this difference did not persist when corrections were made for the higher protein or DNA content of the tumors. To the

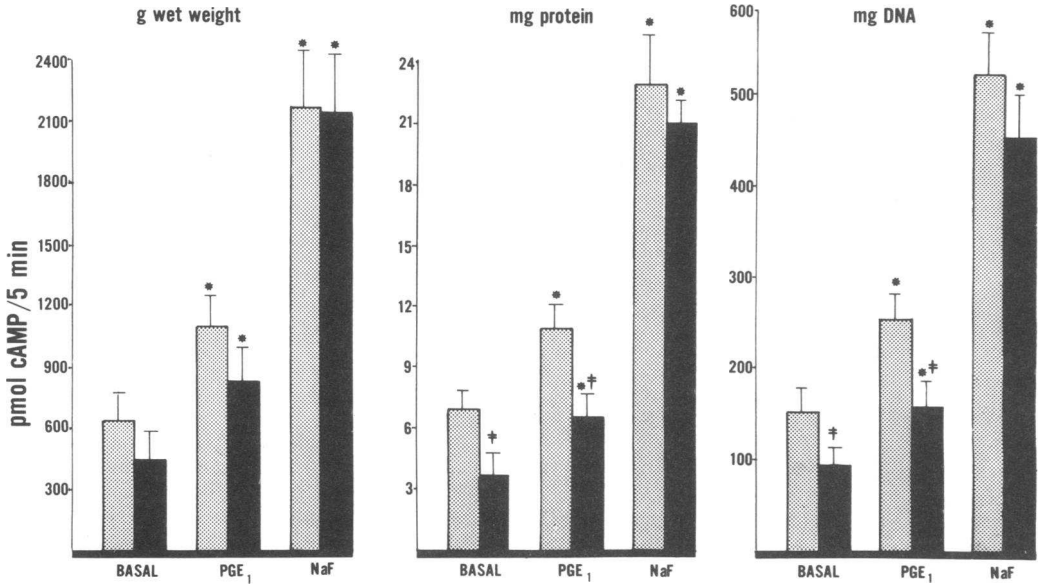

FIGURE 5 Basal, PGE_1- and NaF-responsive adenylate cyclase activities of six carcinomas (solid bars) and adjacent mucosa (stippled bars), expressed on the basis of tissue wet weight, protein, and DNA. Enzyme activity was assayed at 30°C in whole tissue homogenates. Values represent mean±SE of triplicate determinations from six experiments; $*P < 0.025$ comparing PGE_1 or NaF to basal of the same tissue; $‡P < 0.025$ comparing value of tumor and mucosa for the same condition.

extent that the somewhat higher DNA content of the tumors reflected an increase in the cellularity of this tissue, the results would suggest that cellular levels of cGMP were equivalent in the two tissues.

An enhanced rate of cAMP degradation has been implicated in the reduced cAMP content found in some experimentally induced rodent hepatomas (31–33). However, this seemed an unlikely explanation for the reduced cAMP of colonic tumors, since (*a*) the presence of theophylline during in vitro incubations failed to restore cAMP in the tumors to levels found in the surrounding mucosa; and (*b*) significant differences in cAMP-phosphodiesterase activities between the two tissues were not observed. By contrast, the possibility that the lower cAMP content of the carcinomas was due to a reduced rate of nucleotide synthesis was supported by (*a*) the lower levels of cAMP in tumor slices compared to mucosa after incubation of both tissues with maximal doses of PGE_1 with theophylline; and (*b*) the lower basal and PGE_1-responsive adenylate cyclase activities of the tumor homogenates. Na-F responsive cyclase activity of the tumors was comparable to that of the adjacent mucosa. According to current concepts (34), NaF is thought to activate adenylate cyclase system at its catalytic site. In so far as this hypothesis is correct and applicable to the colonic tumors, the reductions in basal and PGE_1-responsive adenylate cyclase activities in this tissue may not be due to a deficiency of the catalytic unit of the enzyme. These reductions could reflect alterations in the plasma membrane affecting the receptor or coupling mechanisms of the cyclase system, or the presence of inhibitors of basal or PGE_1-responsive enzyme activities in the tumor homogenates.

The role, if any, of reduced activity of the adenylate cyclase-cAMP system or of lower cAMP-to-cGMP ratios of the tumors in the pathogenesis of colonic carcinoma remains uncertain. Data from studies of several cell lines in culture are consistent with the possibility that a reduction in cellular cAMP, an increase in cellular cGMP, or both may be invloved in unregulated proliferation of transformed cells. Thus, the cAMP levels of several fibroblast lines were inversely related to their doubling time (15), with faster-growing, non-contact-inhibited, transformed cells containing less cAMP than the nontransformed parent cells (5, 15). In the latter, cAMP rose three- to four-fold when cells reached confluency and growth ceased (15). Exogenous cAMP or agents that raised intracellular levels of cAMP restored contact inhibition and several normal morphologic characteristics to transformed cells (2–4, 14). Similarly, cAMP has been shown to inhibit lymphocyte mitogenesis in vitro, and synchronized cultures of lymphoid cells

have lower cAMP levels during the S and M phases of their growth cycle than during G_1 and G_2 (35, 36). Recent evidence has suggested that cAMP may exert its inhibitory action on cell growth by alterating cell surface properties, possibly through an action on the microtubule system (8, 37). Conversely, cGMP has been reported to stimulate growth of both fibroblasts and lymphocytes in culture (11–13, 16, 38) and increases in cellular cGMP content have been implicated in the initiation of proliferation of these cells (7, 11, 13, 16). Decreased activity of the adenylate-cAMP system and increased cGMP content have also been observed in psoriatic epithelium, whose proliferation is accelerated compared to the adjacent uninvolved skin (39, 40). Such observations are consistent with a pathogenetic role for the reduced cAMP content and cAMP-to-cGMP ratio of colonic carcinomas in the malignant transformation of this tissue.

However, a simple relationship between the cAMP or cGMP content of cells and their conversion to a neoplastic form seems unlikely. In the present study, a wide overlap in the absolute levels of cAMP and cGMP between the tumors and uninvolved mucosa was obvious, with the cAMP content of some carcinomas being twice that of some mucosal samples. Furthermore, no consistent pattern of changes is evident when cAMP or cGMP levels of other tumors of man or experimental animals are compared to those of the parent tissue. Thus, in earlier studies we found higher adenylate cyclase and cAMP levels in adenomas and carcinomas of the human thyroid than in the surrounding uninvolved gland (22, 23). Similarly, while some rat hepatomas have reduced cAMP or increased cGMP (19, 21), in others cAMP has been the same or higher than that of the surrounding liver (18–20). Moreover, under certain experiment conditions, cAMP has been implicated as a positive rather than negative signal for hepatocyte growth (41, 42), and in the rapidly proliferating crypt cells of intestinal mucosa, adenylate cyclase activity has been reported to be higher than that of the more slowly growing superficial cells (43).

Such observations would suggest that absolute levels of cAMP or cGMP are not exclusive determinants of cell growth or malignant transformation. The net impact of cAMP or cGMP on the control of cell proliferation may be modulated by a variety of other factors, including complex alterations in cyclic nucleotide metabolism that could impair expression of normal cellular actions of the nucleotides in transformed cells. In this regard, altered subcellular distributions of adenylate and guanylate cyclase (44, 45), and phosphodiesterase activities (33), abnormal cAMP and cGMP binding capacities and protein kinase regulation (46, 47), and an altered response pattern to hormonal stimuli (20, 35, 48, 49) have now all been observed in neoplastic tissues. The extent to which such changes occur in colonic carcinomas remains to be determined. Further studies along these lines seems indicated, since present evidence indicates that the cAMP and cGMP content of this tumor are altered in a direction that may favor unregulated cell proliferation.

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