Decreased Cerebrospinal Fluid Cyclic Adenosine 3',5'-Monophosphate in Bacterial Meningitis

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The concentration of cyclic adenosine 3',5'-monophosphate (cAMP) in 16 cerebrospinal fluid samples from eight patients with bacterial meningitis due to several different organisms was determined. An age- and sex-matched control group of 12 patients with a variety of acute, noninfectious systemic and neurological diseases was also examined. To quantitate the amount of cAMP, a new, improved radioimmunoassay was used with the ability to measure 2.5×10^{-15} mol of cAMP. The mean concentration of cAMP in the cerebrospinal fluid from patients with meningitis was 0.05 nM, and from patients in the control group it was 1.18 nM. The difference between these two values is statistically significant. The decreased cAMP concentration in the cerebrospinal fluid from patients with bacterial meningitis did not seem to be secondary to metabolism by bacteria or leukocytes, increased enzymatic degradation within the cerebrospinal fluid, or an artifact introduced by the collection and storage procedure. Since the concentration of cAMP in the cerebrospinal fluid is normally found to be within narrow limits and probably reflects intracellular cAMP levels, the results described in this study suggest that interference with cAMP metabolism in central nervous system tissue occurs in bacterial meningitis. This finding seems to be independent of the causative organism and might explain the pathogenesis of selected, neurological manifestations of this disease.

Bacterial meningitis, an infection of the leptomeninges, can be caused by a wide variety of bacteria. Both the systemic and neurological manifestations of acute bacterial meningitis, however, are largely independent of the specific, causative organism. Classical systemic features of infection, such as fever, chills, malaise, and leukocytosis, are present, with variations in this picture occurring at the extremes of age. Infants can appear simply fretful and difficult to feed, and older patients can have a nonspecific, flulike illness (17).

In addition to these systemic signs and symptoms, a variety of manifestations referable to the central nervous system (CNS) occur. Signs of meningeal irritation such as stiff neck and, in children, opisthotonos, as well as manifestations of increased intracranial pressure, such as headache and vomiting, are easily understood, considering the pathology of this disease. However, another spectrum of acute neurological signs is also a prominent part of the clinical picture in bacterial meningitis and yet does not have clear histopathological correlates. In infants, apneic episodes, twitching, seizures in up to 30% of cases, and coma may develop acutely. In adults,

the mental status can progress from delirium in the early stages to drowsiness, stupor, and then coma. The striking feature at necropsy, however, is the minimal amount of necrosis and inflammation that can be present in brain tissue (7). In fulminant fatal cases, at most only slight flattening of the gyri and a few polymorphonuclear leukocytes in sections of the meninges and brain are evident. In patients who die of bacterial meningitis after several days, a more extensive purulent exudate covers the meninges and surface of the brain with some minimal degree of histological encephalitis. The pathophysiological mechanism by which an inflammation in the meninges causes severe clinical neurological abnormalities remains unclear (4).

An avenue of investigation which might resolve this question would involve a study of biochemical changes which might occur during bacterial meningitis. The key role which cyclic adenosine 3',5'-monophosphate (cAMP) plays in a number of intracellular biochemical reactions in the CNS has recently been extensively reviewed by Nathanson (11). Attempts to measure cAMP in the CNS would appear to be a logical first step in approaching this problem.

Previously, a number of other diseases with neurological signs that cannot be explained completely by gross or microscopic lesions have been studied along these lines. In coma secondary to head trauma and intracranial hemorrhage (12), seizures (10), and diffuse brain disease in children causing psychomotor retardation (13), different investigators have described deviations from normal levels in the concentration of cAMP in the cerebrospinal fluid (CSF). The present study was designed to examine whether the concentration of cAMP in patients with acute bacterial meningitis differed from that in patients with other acute neurological and systemic diseases. Quantitation of any changes in bacterial meningitis might be the initial step in delineating biochemical aspects of the pathogenesis of this disease.

MATERIALS AND METHODS

Collection of CSF samples. A total of 34 CSF samples were obtained from 25 patients by lumbar puncture. In all cases, lumbar puncture was performed solely for routine diagnostic reasons and not for the purpose of use in this study. Most of the CSF samples (10 of 16) from patients with bacterial meningitis were frozen within 1 h after collection and stored at -20°C until assayed for cAMP. The rest of the CSF samples from patients with meningitis as well as all from the control group were treated in a routine manner by the hospital chemistry laboratory. This included centrifugation at $800 \times g$ for 20 min to pellet cells, removal of a portion of the supernatant for determination of protein and glucose, and storage of the remaining CSF (0.2 to 5 ml) at 4°C for 1 to 3 days. The samples were then stored at -20°C until assayed for cAMP.

The "meningitis" group consisted only of CSF from patients with clinical findings compatible with a diagnosis of bacterial meningitis in whom the initial lumbar puncture yielded a positive bacterial culture. Samples from patients with other CNS infections (viral, tuberculosis, fungal, or parameningeal) were excluded. A "control" group was matched for age and sex but otherwise was chosen randomly from culture-negative CSF samples from patients with acute neurological or systemic diseases of nonbacterial etiologies. In addition, CSF samples from five patients with no obvious organic disease were designated as "normal." Three of these underwent lumbar puncture because of headache and two because of lumbrosacral pain. Subsequent clinical studies of these patients failed to reveal any organic disease.

In vitro studies. To test for any influences that bacteria might have on cAMP concentrations in CSF, a portion from an overnight growth of *Streptococcus pneumoniae* was diluted 1:10 into samples of CSF. This yielded approximately 10^7 to 10^8 organisms per ml. After incubation for 24 h at 37°C , the bacteria were pelleted at $10,000 \times g$ for 30 min, and the supernatant was assayed for cAMP. To test for any effect of leukocytes on CSF cAMP, a portion from the buffy coat of freshly drawn, heparinized blood was diluted 1:

10 into CSF samples. This resulted in approximately 10^7 to 10^8 leukocytes per ml. After incubation at 37°C for 24 h, the cells were pelleted at $1,000 \times g$ for 10 min, and the supernatant was assayed for cAMP.

Purification of cAMP. The CSF samples were deproteinized by the addition of perchloric acid to a final concentration of 1%, incubation at 0°C for 10 min with periodic mixing, and centrifugation at $10,000 \times g$ for 20 min. The supernatant was decanted, adjusted to pH 7.0 with 6 N KOH, and centrifuged at $10,000 \times g$ for 20 min. The supernatant was further fractionated by ion-exchange chromatography, using a 1-ml column of Bio-Rad AG-1-X8, 200 to 400 mesh, equilibrated with 0.1 N formic acid. After application of the deproteinized sample and washing with 10 ml of 0.1 N formic acid, cAMP was eluted with 10 ml of 2 N formic acid.

Quantitation of cAMP. cAMP was measured by using the method of Steiner et al. (15) modified by Cailla et al. (2). The purified cAMP in 2 N formic acid was evaporated to dryness under reduced pressure, and the residue dissolved in 0.05 M sodium acetate was adjusted to pH 6.2 with glacial acetic acid. Since the antibody used to measure cAMP was generated by using succinylated cAMP (ScAMP) as an antigen, the cAMP purified from the CSF sample was succinylated by making the sample 5% in triethylamine and then adding 0.5 mg of succinic anhydride dissolved in tetrahydrofuran for 10 min at room temperature.

The radioimmunoassay to quantitate the purified ScAMP employed the following reagents from Collaborative Research, Inc. (Waltham, Mass.): ^{125}I -ScAMP iodo-tyrosine methylester (^{125}I -ScAMP-TME); rabbit antibody to 2'-O-succinyl cAMP; sheep anti-rabbit serum; and cAMP standards. A total of 5,000 cpm of ^{125}I -ScAMP-TME was added to all samples, and the ScAMP was precipitated with the double-antibody technique. First, rabbit anti-ScAMP was added in amounts to keep the antigen (ScAMP) in excess over antibody. Then sheep anti-rabbit serum was added in amounts that would precipitate all the rabbit antibody. After 16 h, the immunoprecipitate was pelleted at $1,000 \times g$ for 20 min and washed twice in 0.02 M Na_2PO_4 -0.15 M NaCl, and the radioactivity in the pellet was determined in an Isodyne gamma counter. The percentage of ^{125}I -ScAMP-TME in the immunoprecipitate compared to the supernatant varied inversely with the amount of unlabeled ScAMP. By preparing a standard curve, using known amounts of unlabeled cAMP, which relates the percentage of labeled cAMP bound in the antigen-antibody complex to the known amount of unlabeled cAMP in the reaction tube, an unknown sample of cAMP can be determined by comparison with the curve. The purified ScAMP from the meningitis and control CSF samples were assayed simultaneously along with a series of known amounts of cAMP. All assays were performed in duplicate and were within 10% of each other. Since the radioimmunoassay determines the amount of cAMP present, all results were initially expressed in picomoles. These results were recalculated and expressed as concentrations (nanomolar) based on the total volume of CSF that was present initially in each sample.

Statistical analysis. Statistical significance was determined by the Wilcoxon Rank-Sum test (1). Data

in the tables and figures are given as the mean $\pm$ standard deviation.

RESULTS

Clinical data. Table 1 contains the clinical profile of eight patients with bacterial meningitis and the CSF findings from 16 lumbar punctures. The patients' ages ranged from 6 months to 84 years and exhibited a bimodal distribution—6 months to 3 years and 45 years to 84 years—typical of bacterial meningitis which tends to be a disease of the very young and old (7, 17). The male-to-female ratio was 1:1. The organisms responsible for the meningeal infection reflect the more common bacteria involved in meningitis, *H. influenzae*, *N. meningitidis*, and *S. pneumoniae* (7, 16). Finding three cases of *Listeria monocytogenes* and one of group B streptococcus in a series of this size, however, is unexpected. Patients 1, 2, 5, and 7 had serial lumbar punctures, and only the initial ones yielded bacterial growth. All subsequent CSF samples were sterile, since all patients had been on appropriate antibiotic therapy by the time these additional lumbar punctures were done.

All patients with bacterial meningitis had el-

evated CSF protein (>40 mg/100 ml), ranging from 42 to 685 mg/100 ml. The glucose concentration, normally more than one-half the simultaneous blood glucose, was depressed in all but four samples (patient 1, initial lumbar puncture; patient 7, lumbar punctures from days 1, 4, and 5). A pleocytosis is evident in all samples, with leukocyte counts ranging from 38 to 30,000 cells per mm³, and the characteristic neutrophilic predominance can be seen.

The control group consisted of 12 patients without bacterial meningitis. The clinical data from this group and the findings from 13 lumbar punctures are presented in Table 2. The control population did not consist of "healthy normals" but of patients with a heterogeneous array of nonbacterial neurological and systemic diseases. A control group of this composition minimized the possibility that an alteration in cAMP concentration in the bacterial meningitis group represented a nonspecific response to disease in general.

The cell distribution and sex ratio in the control group were comparable to those in the meningitis group. Two patients in the control group (patients 13 and 16) had CSF findings charac-

TABLE 1. Data from patients with bacterial meningitis

Patient	Age	Sex ^a	Culture	CSF ^b			
				Protein (ml/100 ml)	Glucose/simultaneous blood glucose (mg/100 ml)	Leukocytes/mm ³ ^c	cAMP concn (nM)
1	6 months	M	<i>S. pneumoniae</i>	445	55/88	38 (80)	0.06
			NG	685	5/132	200 (60)	0.06
2	7 months	F	<i>N. meningitidis</i>	180	51/110	5,900 (100)	0.09
			NG	42	100/NA	1,300 (70)	0.01
3	2 years	F	<i>H. influenzae</i>	96	30/96	950 (90)	0.02
4	3 years	F	<i>H. influenzae</i>	152	63/NA	345 (88)	0.15
5	45 years	M	<i>L. monocytogenes</i>	320	25/265	923 (90)	0.009
			NG	422	65/190	270 (95)	0.008
6	55 years	M	<i>L. monocytogenes</i>	80	40/98	250 (90)	0.004
7	61 years	M	Group B <i>Streptococcus</i>	270	150/450	30,000 (100)	0.07
			[1] NG	245	210/350	10,000 (90)	0.06
			[2] NG	NA	NA	NA	0.04
			[4] NG	134	136/222	6,400 (NA)	0.03
			[5] NG	212	114/214	7,200 (NA)	0.08
			[12] NG	NA	84/177	1,600 (NA)	0.07
8	84 years	F	<i>L. monocytogenes</i>	125	60/165	221 (90)	0.007

^a Values in brackets represent number of days after initial lumbar puncture. M, Male; F, female.

^b NG, No growth; NA, not available.

^c Values in parentheses indicate percent neutrophils.

TABLE 2. Data from patients with nonbacterial neurological or systemic disorders^a

Patient	Age	Sex	Primary diagnosis	Protein (mg/ 100 ml)	CSF			
					Glucose/ simultaneous blood glucose (mg/100 ml)	Leukocytes/ mm ³	Erythrocytes/ mm ³	cAMP (nM)
9	1 month	M	Fever	34	64/NA	2	300	0.71
				196	63/NA	NA	Grossly bloody	1.10
10	2 years	M	Seizure	27	87/NA	1	1	0.52
11	2 years	F	Seizure	26	65/92	0	0	1.00
12	14 years	F	Headache	37	71/102	1	6	0.56
13	56 years	F	Brain tumor	81	70/110	80 (100% lym- phocytes)	0	0.25
14	56 years	M	Uremia	24	80/105	0	0	2.16
15	57 years	M	Alcoholism	38	152/NA	0	0	5.44
16	62 years	M	Epidural tu- mor	176	78/98	85 (80% lympho- cytes)	0	0.38
17	63 years	F	Fever	42	105/130	2	2	0.26
18	72 years	M	Stroke	121	67/NA	0	250	0.49
19	75 years	M	Stroke	68	64/112	0	29	0.38
20	76 years	F	Stroke	95	NA	1	50	1.57

^a NA, Not available; M, male; F, female.

teristic of an "aseptic meningitis" with an elevated protein concentration, normal glucose concentration, and moderate lymphocytic pleocytosis. In both cases, a parameningeal tumor caused these changes. The clinical impression in both CSF samples from patient 9 was that a "traumatic" lumbar puncture resulted in the "bloody" sample with an elevated protein. The neurological disease in patients 18, 19, and 20 most likely caused the presence of red blood cells and the elevation of protein concentration.

CSF cAMP levels. The cAMP concentration in the bacterial meningitis group ranged from 0.004 to 0.15 nM and in the control group from 0.25 to 5.44 nM (Tables 1 and 2). These results are graphically illustrated in Fig. 1. In the meningitis samples, the mean was 0.05 nM, and in the controls it was 1.18 nM. The difference between the two groups is statistically significant ($P < 0.001$). In fact, no overlap can be seen, and a value slightly greater than 0.1 nM separates the two populations from each other.

The possibility that other factors besides bacterial meningitis might be associated with decreased CSF cAMP levels was investigated by utilizing data derived from Tables 1 and 2 and information from the patients' charts. No correlation could be demonstrated within either group between the concentration of cAMP and any of the following parameters: CSF glucose, protein, or cells; opening pressure during lumbar puncture; the patient's age, sex, temperature, medications, or concomitant diseases; length of time between lumbar puncture and the assay; or whether the CSF sample was immediately fro-

FIG. 1. Concentrations of cAMP in the CSF from patients with meningitis and patients in a control group. Each point represents the average of two determinations done on a single CSF specimen. Bars represent the mean $\pm$ standard deviation.

zen after lumbar puncture or stored at 0 to 4°C for 1 to 3 days. All patients were alert, and none died acutely of their illness except for the three patients with *Listeria* meningitis. These were

the only patients who went into a coma and died acutely, and their CSF cAMP concentrations were the lowest in the series (0.004 to 0.009 nM). The number of patients in this group was too small to determine whether this association between coma, mortality, and markedly depressed cAMP levels was statistically significant.

In this study, to make the comparison with acute bacterial meningitis more valid, a population with other acute, serious neurological and systemic diseases was used as a control. To insure that this choice of a control group did not bias the conclusions of this study, cAMP was measured in the CSF from five patients with no obvious organic disease and found to average 2.3 nM (range, 0.96 to 5.16 nM). Although slightly higher than the average concentration of cAMP in the nonmeningitis, acutely ill group, this difference is not statistically significant.

In vitro studies. A number of in vitro studies were designed to explore the possibility that alterations in cAMP concentration might be secondary to spontaneous hydrolysis during storage, enzymatic hydrolysis due to phosphodiesterases, or metabolism by leukocytes and/or bacteria. Five different samples of CSF from patients without bacterial meningitis were divided into five equal portions. Each of the aliquots was treated as described in Table 3 and in Materials and Methods, and was then assayed for cAMP. None of the procedures resulted in a statistically significant change in cAMP concentration. The slightly lower level observed after 3 days at 4°C might be due to minimal nonenzymatic hydrolysis; this might also explain the slight decrease after incubation with bacteria, although a low-grade catabolic effect by the bacteria cannot be ruled out. Since leukocytes have an active cAMP metabolism (16), the moderate elevation resulting from incubation with leukocytes could have been secondary to release of cAMP from dead or lysed cells. The inability of CSF from patients with bacterial meningitis to lower the cAMP level in control samples provides strong evidence against the presence of phosphodiesterases as well as other unidentified interfering factors or cAMP-degrading factors in CSF from patients with bacterial meningitis.

DISCUSSION

Recent studies of cyclic nucleotide involvement in the physiology of the central nervous system have led to an increasing awareness of the importance of cAMP-mediated processes. A number of investigators have postulated that cAMP is involved in: (i) regulation of neurotransmitter synthesis; (ii) the mechanism of some neurotransmitter-induced permeability

TABLE 3. *In vitro* studies examining the effects on cAMP concentration of storage temperature, bacteria, leukocytes, and CSF from patients with bacterial meningitis^a

CSF sample conditions	cAMP concn (nM)
Stored at -20°C	1.38 ± 0.17
Stored at 4°C for 3 days	1.08 ± 0.27
Incubated with bacteria for 24 h at 37°C	1.14 ± 0.21
Incubated with leukocytes for 24 h at 37°C	2.84 ± 0.96
Incubated with an equal amount of CSF from a patient with meningitis for 24 h at 37°C	1.44 ± 0.26

^a Each value represents mean ± standard deviation for five samples.

changes; (iii) control of certain intracellular movements; (iv) effects on trophic developmental processes; and (v) regulation of cerebral carbohydrate metabolism (11). Compatible with its central role in a number of these intracellular CNS functions has been the demonstration of a 10-fold higher concentration of cAMP in central nervous tissue compared with most nonneural tissue (11, 18).

cAMP, a compound synthesized intracellularly, can be passively or actively extruded from cells (3, 8). Sebens and Korf have shown an increase in CSF cAMP after intraventricular injection of adrenergic agents which also increase cAMP in brain slices (14). Since systemically injected cAMP does not penetrate into the CSF, the cAMP found in the CSF is of central origin (14). Based on these reports, CSF cAMP seems to reflect intracellular cAMP, an assumption supported by a number of clinical papers which have described alterations in CSF cAMP concentration coincident with acute and chronic diseases of the CNS. A group of investigators from Finland has described an elevation of CSF cAMP after seizures (10) and cerebral infarction (5). This same group has also reported elevated CSF cAMP in children with bacterial meningitis (6). (An explanation for the discrepancy between their results and ours probably involves methodological differences discussed below.) Rudman and co-workers have reported low CSF cAMP levels in children with retarded psychomotor development caused by diffuse brain disease (13) and also in prolonged coma secondary to head trauma and intracranial hemorrhage (11). In the latter study, the concentration of CSF cAMP correlated with the stage of coma, dropping to its lowest level during stage IV coma (complete unresponsiveness) and rising concomitantly with clinical improvement in mental status. These reports establish that cAMP concentration in

the CSF is affected by a variety of neurological disorders.

The results of this study indicate a significant decrease in the concentration of cAMP in the CSF from patients with bacterial meningitis as compared to patients in a control group. The controls in this paper had a mean cAMP concentration of 1.4 nM (Fig. 1). The studies discussed above, however, reported an average of 21 nM and 25 nM cAMP in the CSF of controls (10, 12). Most likely, this reflects methodological differences in measuring cAMP. The assay which is used can clearly lead to different "normal" values, since Kassen and Kagen, employing even another assay, reported normal CSF levels of cAMP to be 7.3 ± 1.4 nM (8).

The present study employed an improved radioimmunoassay that involves two additional steps not previously used in measuring CSF cAMP: (i) purification of cAMP from the CSF, using ion-exchange chromatography; and (ii) succinylation of the purified product. Although the purification procedure could lead to a loss of small amounts of cAMP, the ultimate result of the purification and succinylation is an increased specificity and reproducibility as well as a 100-fold increase in sensitivity (2); 2.5×10^{-15} mol of cAMP can be routinely determined with this method. The basis for this greatly increased sensitivity is that the cAMP antibody, generated to a succinylated AMP immunogen, has a greater affinity for the succinylated than for the native cAMP. This assay permitted determination of values in the 0.005 to 0.5 nM range which were below the level of detection reported in earlier papers.

Since a low CSF cAMP concentration could result from decreased synthesis and/or increased loss, a number of possibilities need to be explored in considering the depressed levels in bacterial meningitis. Although bacterial and leukocyte cells both have an active cAMP metabolism (16), it seems unlikely that they caused the low CSF cAMP concentration. Eight of 15 CSF samples from patients with meningitis had no bacterial growth since the patients were treated with appropriate antibiotics at the time of lumbar puncture. Despite a wide variation in the leukocyte count extending over three orders of magnitude (38 to 30,000 cells/mm³), no correlation between cAMP and leukocyte count could be demonstrated. The *in vitro* studies (Table 3) provide further evidence against the possibility that leukocytes or bacteria have catabolized cAMP. Similarly, spontaneous or enzymatic hydrolysis seems unlikely, based on the *in vitro* data and also since phosphodiesterase activity has been undetectable in other cases of

decreased CSF cAMP (12).

Although increased transport out of the CSF cannot be ruled out, based on the data in this and other studies, the decreased concentration of cAMP in the CSF of patients with bacterial meningitis most likely results from a disorder of cAMP metabolism in CNS tissue leading to a decreased intracellular concentration of cAMP. Adenyl cyclase, which controls the synthesis of cAMP, responds to a large number of extracellular "signals" such as hormones, depolarizing stimuli, biogenic amines, and a number of different ions (11). This responsiveness to environmental influences and the location at the plasma membrane would seem to expose adenyl cyclase to extracellular pathological processes. Considering bacterial meningitis as such a process, a speculative sequence of events might involve a decrease in adenyl cyclase activity initially. The bacterial infection could cause this decrease directly, or indirectly, by interfering with or perturbing any of the extracellular physiological mediators of adenyl cyclase activity. Since this study reported CSF cAMP changes in meningitis due to five different bacteria, it seems unlikely that a specific toxin inactivates adenyl cyclase. More probable is the involvement of a product of bacterial metabolism or a common component of the bacterial cell wall. The alteration in adenyl cyclase activity would then lead to a decrease in cAMP synthesis and a disturbance in any of the biochemical reactions mediated by cAMP. The change in intracellular cAMP would be reflected in a corresponding decrease in CSF cAMP. (An alternative scheme could involve a stimulation of intracellular phosphodiesterases instead of an inhibition of adenyl cyclases as the link between a pathological process and altered intracellular cAMP levels.) An important feature of this admittedly hypothetical construct is that it outlines a sequence of cAMP-mediated events which could account for the appearance of serious clinical signs and symptoms without the appearance of correspondingly serious morphological abnormalities.

Alterations in cAMP do not appear to be a nonspecific response to disease in general. In this study, the controls had a significantly higher CSF cAMP and yet consisted of a random sampling of patients with serious neurological and system disorders (Table 2). The concentration of CSF cAMP in this acutely ill group was not significantly different than in the concentration in healthy normals. It would seem that changes in CSF cAMP concentrations occur in some but not all pathological states, although the nature of the specificity remains unclear. No data are available, for example, on the CSF cAMP find-

ings in viral, fungal, or tuberculous or other aseptic meningitidis, and such investigations are beyond the scope of this paper. However, only after such data are collected and analyzed can any consideration be given to using CSF cAMP concentrations as a diagnostic aid. At this point, the measurement of CSF cAMP remains an investigative attempt to gain insight into the pathogenesis of bacterial meningitis at a molecular level.

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LITERATURE CITED

- Bradley, J. F. 1978. Distribution-free statistical tests, p. 105-115. Prentice-Hall, Inc., Englewood Cliffs, N.J.
- Cailla, H. L., M. S. Racine-Weisbuch, and M. A. De-laage. 1973. Adenosine 3',5' cyclic monophosphate assay at 10^{15} mole level. *Anal. Biochem.* **56**:394-407.
- Door, B. J., M. M. Bashor, N. Spitzer, R. C. Mowe, and M. H. Saier. 1975. Regulation of adenosine 3',5'-monophosphate efflux from rat glioma cells in culture. *J. Biol. Chem.* **250**:4371-4372.
- Harter, D. H., and R. G. Petersdorf. 1959. A consideration of the pathogenesis of bacterial meningitis: review of experimental and clinical studies. *Yale J. Biol. Med.* **32**:280-309.
- Heikkinen, E. R., V. V. Myllyla, E. Hokkanen, and H. Vapaatalo. 1976. Cerebrospinal fluid concentration of cyclic AMP in cerebrovascular diseases. *Eur. Neurol.* **14**:129-137.
- Heikkinen, E. R., S. Simila, V. V. Myllyla, and H. Vapaatalo. 1975. Cyclic adenosine 3',5'-monophosphate concentration and enzyme activities of cerebrospinal fluid in meningitis in children. *Z. Kinderheilkde.* **120**:243-250.
- Hoepflich, P. D. 1977. Acute bacterial meningitis, p. 889-901. *In* P. D. Hoepflich (ed.), *Infectious diseases*. Harper & Row, Publishers, Inc., Hagerstown, Md.
- Kassen, S. S., and L. J. Kagen. 1978. Elevated levels of guanosine 3',5'-cyclic monophosphate (C-GMP) in systemic lupus erythematosus. *Am. J. Med.* **64**:732-741.
- Lindl, T., and H. Cramer. 1974. Formation, accumulation and release of adenosine 3',5'-monophosphate induced by histamine in the superior cervical ganglion of the rat in vivo. *Biochim. Biophys. Acta* **343**:182-191.
- Myllyla, V. V., E. R. Heikkinen, H. Vapaatalo, and E. Hokkanen. 1975. Cyclic AMP concentration and enzyme activities of cerebrospinal fluid in patients with epilepsy or central nervous system damage. *Eur. Neurol.* **13**:123-130.
- Nathanson, J. A. 1977. Cyclic nucleotides and central nervous system function. *Physiol. Rev.* **57**:157-256.
- Rudman, D., A. Fleischer, and M. H. Kutner. 1976. Concentration of 3',5' cyclic adenosine monophosphate in ventricular cerebrospinal fluid of patients with prolonged coma after head trauma or intracranial hemorrhage. *N. Engl. J. Med.* **295**:635-638.
- Rudman, D., M. S. O'Brien, A. S. McKinney, J. C. Hoffman, Jr., and H. E. Patterson. 1976. Observations on the cyclic nucleotide concentrations in human cerebrospinal fluid. *J. Clin. Endocrinol. Metab.* **42**:1088-1097.
- Sebens, J. B., and J. Korf. 1975. Cyclic AMP in cerebrospinal fluid. Accumulation following probenecid and biogenic amines. *Exp. Neurol.* **46**:333-344.
- Steiner, A. L., D. M. Kipnis, R. Utiger, and C. Parker. 1969. Radio-immunoassay for the measurement of adenosine 3',5'-cyclic phosphate. *Proc. Natl. Acad. Sci. U.S.A.* **64**:367-373.
- Sutherland, E. W. 1971. The intracellular level of cAMP, p. 29-35. *In* G. A. Ribonsin, R. W. Butcher, and E. W. Sutherland (ed.), *Cyclic AMP*. Academic Press Inc., New York.
- Swartz, M. N., and P. R. Dodge. 1975. Bacterial meningitis—a review of selected aspects. I. General clinical features, special problems and unusual meningeal reactions mimicking bacterial meningitis. *N. Engl. J. Med.* **272**:725-731, 779-787, 842-848, and 898-902.
- Weiss, B., and L. H. Greenberg. 1975. Cyclic AMP and brain function: effects of psychopharmacologic agents on the cyclic AMP system, p. 269-320. *In* *Cyclic nucleotides in disease*. University Park Press, Baltimore.