Proc. Natl. Acad. Sci. USA
Vol. 74, No. 6, pp. 2194–2198, June 1977
Chemistry

Conformational basis for the activation of adenylate cyclase by adenosine

(Ara-adenine/2′-deoxyadenosine/cAMP/immunodeficiency/cardiovascular)

D. L. MILES, D. W. MILES, AND H. EYRING

Chemistry Department, University of Utah, Salt Lake City, Utah 84112

Contributed by H. Eyring, February 28, 1977

ABSTRACT **The ability of adenosine to stimulate adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] and increase adenosine 3′:5′-cyclic monophosphate (cAMP) levels has important biochemical consequences. These include the suppression of immune responses and cardiovascular effects. Recent investigations involving the ability of adenosine and adenosine analogs to stimulate adenylate cyclase provided experimental data that appear to be correlated with the ability of adenosine and analogs of adenosine to exist in the glycosidic high *anti* conformation. 9-β-D-Arabinofuranosyladenine, which is not stable in the high *anti* conformation, is inactive as a stimulator of adenylate cyclase. 2′-Deoxyadenosine is also not stable in the high *anti* conformation but its instability may be significantly decreased by intramolecular adjustments promoted by receptor or active site interactions. 2′-Deoxyadenosine does not activate adenylate cyclase in lymphocytes when ATP is the substrate but is able to activate adenylate cyclase when 2-fluoro ATP is the substrate. The inability of certain analogs of adenosine, with bulky groups substituted for hydrogen at the 8 position of the adenine base, to activate adenylate cyclase and increase either lymphocyte or cardiac cell cAMP levels is consistent with the designation of the high *anti* conformation as being the conformation required for the activation of adenylate cyclase. An understanding of the glycosidic conformation required by the extracellular adenosine receptor of the adenosine molecule provides the basis for designing nucleoside analogs of adenosine that will exert a desired effect on cAMP levels. The avoidance of unwanted immunosuppressive or cardiotoxic effects can be arranged by structural changes that prohibit the high *anti* conformation.**

There are several aspects to the toxicity associated with an excess of adenosine (1–11). The enzymes adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) and adenosine kinase (ATP:adenosine-5′-phosphotransferase, EC 2.7.1.20) compete for adenosine. At higher concentrations of adenosine, adenosine deaminase dominates (11–14) and protects the body from the effects of an excess of adenosine. One of these effects is the suppression of immune responses associated with the activation by adenosine of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] which increases cAMP levels (15, 16). Adenosine stimulation of adenosine 3′:5′-cyclic monophosphate (cAMP) production is attributed to the interaction of adenosine with a receptor for adenosine on the external cell surface (15, 17–24). Immunodeficiency diseases have been associated with the loss of adenosine deaminase activity (5, 25–32).

The coronary vasodilator activity of adenosine has recently been reviewed (33, 34). The cardiovascular effects of adenosine are potentiated by inhibitors of either adenosine deaminase or the uptake of adenosine by the heart (35–42), both of which increase extracellular adenosine concentrations which stimulates adenylate cyclase.

Factors, such as the loss of adenosine deaminase activity, that increase adenosine levels result in increased cAMP levels which are responsible for the immunosuppressive (5, 15) and cardiovascular (33–42) effects of adenosine. In both situations the action of adenosine at a receptor on the external cell surface (15, 17–24) appears to require that adenosine be oriented in the glycosidic high *anti* conformation. This conclusion is suggested by the correlation between the conformational properties of adenosine and adenosine analogs and their capacities to stimulate adenylate cyclase.

Abbreviations: cAMP, adenosine 3′:5′-cyclic monophosphate; Ara-adenine, 9-β-D-arabinofuranosyladenine; Ara-HSR, arabinosyl-6-mercaptopurine.

CALCULATIONS

In order to calculate the conformational differences among adenosine, 2′-deoxyadenosine, and 9-β-D-arabinofuranosyladenine (Ara-adenine) we used the iterative extended Huckel theory (IEHT) method (43). The starting coordinates for the calculations were taken from the crystal structures of these molecules (44–46). The conformational definitions that we will use are shown in Fig. 1. The high *anti* conformation corresponds to dihedral angles between 75° and 165°. Fig. 2 shows the variation in total energy of adenosine with rotation about the glycosidic (C1′–N9) bond. The entire high *anti* conformation range is energetically accessible to adenosine. Calculations that we have done with other crystal forms of adenosine indicate that the conformational energy profile obtained by theoretical calculations on a rigid molecule are modified by the starting coordinates or crystal structure chosen for the calculation. A dramatic example of this has been illustrated by calculations on the two crystal forms of ribavirin (47, 48). In general, the high *anti* conformation in adenosine can be considered as being slightly higher in energy than the *anti* conformation for most of the probable arrangements of atomic coordinates.

In Fig. 3 is the result of a calculation on Ara-adenine. Ara-adenine is unstable in the high *anti* conformation (unpublished data), which accounts for its inactivity as a substrate for both adenosine kinase and purine-nucleoside phosphorylase (purine-nucleoside:orthophosphate ribosyltransferase, EC 2.4.2.1).

Fig. 4, from a calculation on 2′-deoxyadenosine, indicates that this molecule is unstable throughout the high *anti* conformation range. This instability is caused by the conformational adjustments produced by removal of the 2′-OH group that increase destabilizing interactions between C2′ or H2′ and H8. The high *anti* conformation is denied to arabinosyl purines (unpublished data) because adjustments of sugar puckering or the attitude of the base relative to the ribose moiety are unable to decrease significantly the steric interactions between the 2′-OH group and the base moiety that occur in the high *anti* conformation range. Optimizing intramolecular or intermolecular adjustments are unable to stabilize the high *anti* conformation in Ara-adenine. Adjustment of ribose puckering and the degree of tilt between the base and ribose moieties by en-

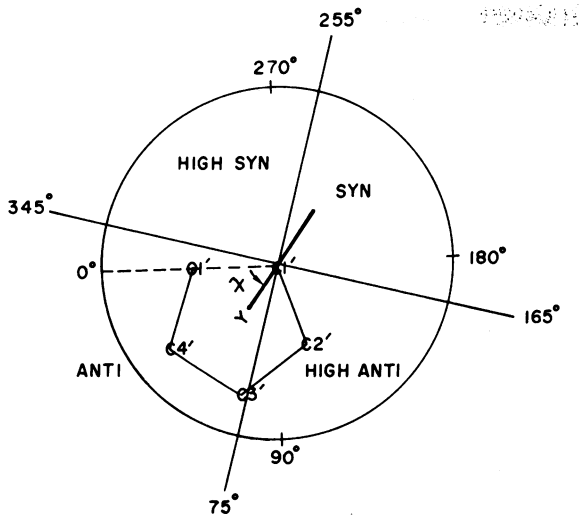

FIG. 1. Definition of glycosidic conformational ranges. A dihedral angle of 0° corresponds to a *cis* planar arrangement of O1′–C1′–N9–Y. The atom Y in this diagram corresponds to C8 of the base moiety of Ara-adenine, 2′-deoxyadenosine, and adenosine. A positive rotation about the glycosidic C1′–N9 bond is a clockwise rotation of the far part of the molecule when looking along the C1′–N9 bond.

zyme or receptor interactions with 2′-deoxyadenosine have the potential of decreasing the instability of the high *anti* conformation. Calculations using the coordinates of Ara-adenine (46) with the 2′-OH group replaced by a hydrogen result in a dramatic decrease of the high *anti* barrier (unpublished data). Certain structural modifications of 2′-deoxyadenosine are apparently able to counteract the conformational effects of removal of the 2′-OH group that increase the instability of the high *anti* conformation.

DISCUSSION

The correlation between the calculated glycosidic properties of adenosine and adenosine analogs and their experimentally established propensities to activate adenylate cyclase is suggested by the following.

1. Ara-adenine, 2′-deoxyadenosine, and 8-bromoadenosine are unable (15, 49) to increase lymphocyte cAMP levels. These molecules are unstable in the high *anti* conformation. However, 2′-deoxyadenosine may be stabilized in the high *anti* conformation through other structural changes or through enzyme–substrate or receptor–substrate interactions with some enzymes or receptors. The activity of 2′-deoxyinosine as a substrate for purine nucleoside phosphorylase and the inactivity of 2′-deoxyadenosine as a substrate for adenosine kinase, both of which require a high *anti* conformation of the substrate (unpublished data), is evidence that stabilizing interactions occur between 2′-deoxyinosine and purine nucleoside phosphorylase.

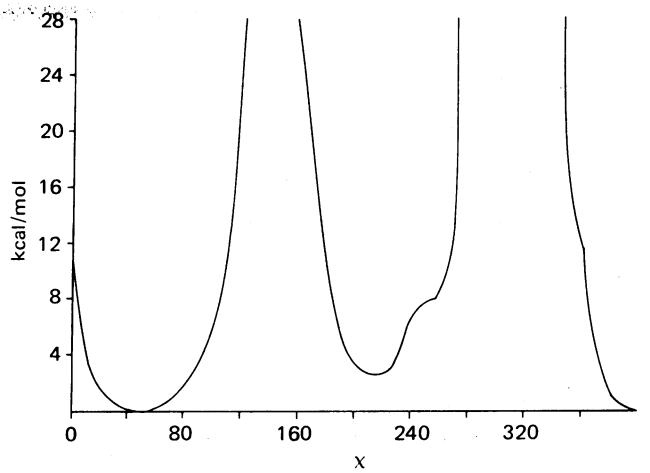

FIG. 3. Variation in total energy plotted against dihedral angle for Ara-adenine.

The adenylate cyclase receptor is apparently modified by the binding of 2-fluoro ATP so that 2′-deoxyadenosine is able to activate adenylate cyclase when 2-fluoro ATP is the substrate but not when ATP is the substrate (15). In contrast, Ara-adenine remains unstable in the high *anti* conformation in spite of the conformational effects of either modification of the substrate at other locations in the molecule or interactions of Ara-adenine with enzyme active sites or membrane surface receptors. Ara-adenine is unable to act as a substrate for purine nucleoside phosphorylase that can accept 2′-deoxyadenosine as a substrate. Ara-adenine is unable to activate adenylate cyclase when 2-fluoro ATP is the substrate but 2′-deoxyadenosine is able to do so. The removal of the 2′-OH group in adenosine destabilizes the high *anti* conformation and this decreases the probability of the proper alignment of the substrate at the active site of either an enzyme or a receptor that requires a molecule bound in the high *anti* conformation. That this instability of the high

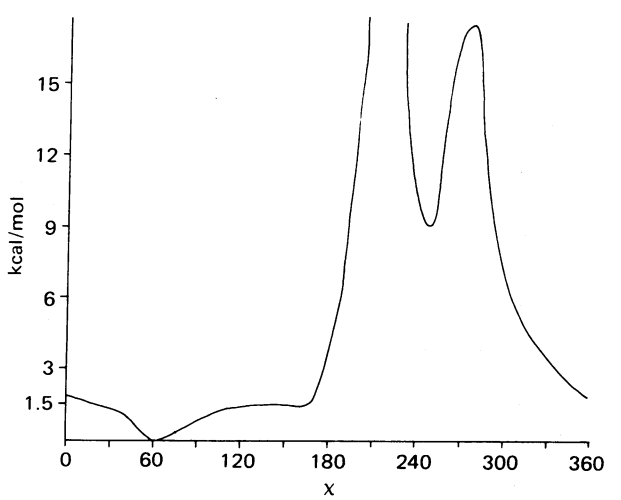

FIG. 2. Variation in total energy plotted against dihedral angle (χ) for adenosine.

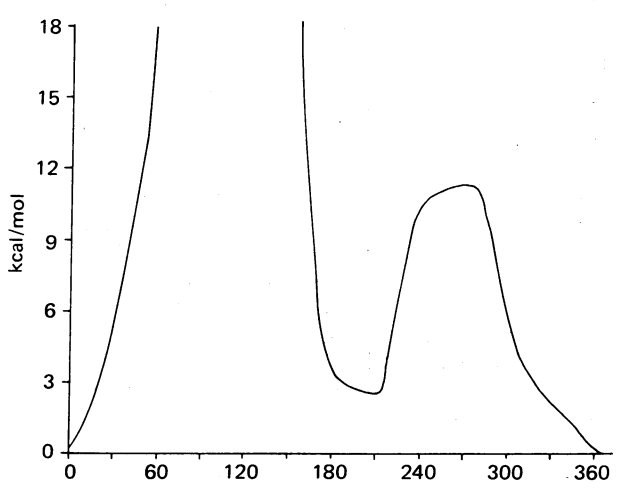

FIG. 4. Variation in total energy plotted against dihedral angle for 2′-deoxyadenosine.

anti conformation in 2′-deoxyadenosine can be overcome by interactions between the adenosine receptor and 2′-deoxyadenosine is demonstrated by the ability of 2′-deoxyadenosine to activate adenylate cyclase when 2-fluoro ATP is the substrate but not when ATP is the substrate. This implies that the conformational adjustments that occur at the adenylate cyclase active site between the active site and a bound substrate influence the conformative response between the adenosine receptor and 2′-deoxyadenosine.

2. 8-Aminoadenosine and 8-bromoadenosine are unable to stimulate cAMP accumulation in guinea pig ventricular slice preparations (14). The inactivity of ribavirin derivatives with methyl, amino, or chloro substituents at the 5 position (50) has been attributed to the destabilization of the high *anti* conformation required for the inhibition of IMP dehydrogenase (IMP:NAD^+ oxidoreductase, EC 1.2.1.14) by these purine analogs (48; unpublished data). Similar structural modifications in adenosine that rule out the high *anti* conformation required for activity, but not the *anti* conformation (51; unpublished data), are inactive as stimulators of adenylate cyclase (37) because these analogs are unstable in the high *anti* conformation. The adenosine analogs 8-aminoadenosine and 8-methylaminoadenosine monophosphates have recently been shown to be stable in the *anti* conformation in solution (51).

3. GTP in the presence of hormones activates adenylate cyclase (52–54). Analogs of GTP such as 3′-deoxy GTP and 5′-guanylyl imidadodiphosphate [Gpp(NH)p] stimulate adenylate cyclase with or without the intervention of a hormone binding to the membrane (55–57). This suggests that a conformation readily accessible to either of these analogs requires the intervention of a hormone-cell surface interaction to stabilize the same conformation in GTP that is necessary for the activation by GTP of adenylate cyclase. The activation of adenylate cyclase at either the GTP receptor or the adenosine receptor requires a properly oriented nucleoside or nucleotide. The inactivity of certain adenosine analogs such as Ara-adenine as stimulators of adenylate cyclase is simply related to their inability to assume the conformation or orientation specified by the receptor.

The lack of immunosuppressive properties in Ara-adenine (58–62) may be attributed to its failure to stimulate adenylate cyclase (15). The character and intensity of immune responses are mediated by cAMP (63–67). The failure of Ara-adenine to depress immune responses (58–62) is due to the instability of Ara-adenine in the high *anti* conformation required for stimulation of adenylate cyclase. The synergistic relationship between Ara-adenine and interferon (62) is possible only because Ara-adenine is incapable of activating adenylate cyclase. Ribavirin, which is stable in the high *anti* conformation (48), does not induce interferon production (68). High levels of cAMP block the production of interferon by sensitized thymus-derived cells (63–67, 69). Interactions between thymus-independent and thymus-derived cells (70) or thymus-derived cell populations (71, 72) that affect immune suppression may be an indication that such interactions affect adenosine levels. The inhibition of rat liver 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) in rapidly proliferating tissue such as Yoshida sarcoma (73), of lymphocyte 5′-nucleotidase in chronic lymphocytic leukemia patients (74), and of 5′-nucleotidase in rat brain by methylxanthines (75) will act to decrease cAMP levels by decreasing the concentration of adenosine. There is an increased incidence of *de novo* tumors in individuals with primary immune deficiency diseases (76) and in patients whose immune system has been deliberately suppressed (77). The variations in adenosine deaminase activity, which disposes of adenosine and blocks the stimulation of adenylate cyclase by adenosine to increase cAMP levels and modify immune responses, seems to be correlated in leukemias (78, 79) with the formation of adenosine by 5′-nucleotidase. The loss of 5′-nucleotidase activity in chronic lymphocytic leukemia (74) is associated with low or normal levels of adenosine deaminase activity (80). The ability to selectively modify either cell-mediated or humoral immunity may be of benefit in the treatment of tumors (65). An increase in cAMP is detrimental to cell-mediated immune responses but may stimulate antibody formation (66) which can interfere and enhance tumor growth (67).

The immunosuppressive activity of arabinosyl-6-mercaptopurine (Ara-HSR), which is not converted to the nucleotide form (81; unpublished data), has been attributed to the ability of this nucleoside to block DNA synthesis through inhibition of the conversion of cytidine diphosphate to deoxycytidine diphosphate by NADH (82).

Aberrations in cyclic nucleotide metabolism appear to characterize a wide range of disease states (83). The immunosuppressive effects found in various infections may facilitate proliferation and metastasis of neoplastic cells or predispose to other infections (84). The removal of unwanted immunosuppressive effects from anticancer agents, as well as improvement in their selectivity, may be done on a common conformational rationale. The preclusion of the high *anti* conformation in Ara-adenine is the basis for its antiviral selectivity (unpublished data) and has been shown here also to be an explanation, consistent with experimental data, for its lack of immunosuppressive properties. The use of cytotoxic agents as anticancer agents (85) has several disadvantages. These disadvantages are based on a failure of the agent to discriminate between tumor cells and normal cells. Adverse mutagenic, carcinogenic, teratogenic, and immunosuppressive effects are associated with most nucleoside analogs considered for use as antitumor agents. These unwanted effects are generally realized after the nontoxic nucleoside is converted to a toxic nucleotide. The minimal toxicity associated with Ara-adenine (58–62) has been attributed to its inability to act as a substrate for either adenosine kinase or purine nucleoside phosphorylase because of the instability of Ara-adenine in the high *anti* conformations required by these enzymes (unpublished data). These enzymes represent the primary mechanisms for converting nontoxic purine nucleoside analogs to a toxic nucleotide form. The residual toxicity to KB cells (86) associated with Ara-adenine may be attributed to its ability to act as a substrate (87) for deoxycytidine kinase (NTP:deoxycytidine 5′-phosphotransferase, EC 2.7.1.74). Ara-HSR, which is an inhibitor of deoxycytidine kinase (87), is not toxic to KB cells (86), presumably because it is not converted to the toxic nucleotide form (88) by either purine (81) or pyrimidine (87) enzymes. Ara-HSR is also unstable in the high *anti* conformation and consequently is not a substrate for adenosine kinase or purine nucleoside phosphorylase. The essentially equal abilities of ara-HSR, which is not toxic to KB cells, and 2′-deoxy-6-mercaptopurine ribonucleoside, which is toxic to KB cells, to increase the life-span of mice infected with L1210 leukemia cells (86) indicates that the desirable separation of cytotoxicity and antitumor properties can be effected. This separation requires that all pathways leading to the conversion of a nontoxic nucleoside analog to a toxic nucleotide analog be either structurally or conformationally prohibited in the body as a whole but that the conversion be allowed in the disease state.

The absence of immunosuppressive properties in adenosine analogs depends on structural changes that will maintain in-

stability in the high *anti* conformation and prevent the analog from assuming the high *anti* conformation required in an adenosine analog for adenylate cyclase activation. Immunosuppressive effects associated with cytotoxicity (82) are avoided when general cellular toxicity is eliminated.

The cardiotoxicity (33–42) of adenosine and adenosine analogs may also be avoided by preventing stability of these analogs in the high *anti* conformation. The 2-chloro and 2-fluoro adenosines decrease both blood pressure and heart rate whereas 2-thioalkyl analogs of adenosine decrease blood pressure but increase the heart rate of anesthetized dogs (42). The cardiotoxicity of analogs of adenosine that is attributable to their resistance to deamination or phosphorylation may be avoided by structural changes that destabilize the high *anti* conformation. The reason for the separation of vasodilatory and negative chronotropic actions of adenosine analogs is not known but it may have a conformational basis. Interactions between adenosine analogs having bulky substituents at the 2 position of the adenine base and the adenosine receptor site may disallow the high *anti* conformation at the postulated cardiac receptor (42) but still allow the high *anti* conformation at the postulated vascular smooth muscle receptor (42).

The increase in cAMP levels and their wide ranging effects (83) induced by adenosine depends on the stimulation of adenylate cyclase by adenosine acting at an extracellular site which requires that adenosine is bound to the receptor in the high *anti* conformation. The inability of Ara-adenine to stimulate adenylate cyclase and the transformation of 2′-deoxyadenosine from an inactive to a moderately active stimulator of this enzyme, when it occurs, appears to be correlated with their glycosidic conformational properties. The instability of the high *anti* conformation in 2′-deoxyadenosine may be sufficiently decreased by structural modifications or by intermolecular interactions to enable some population of the high *anti* conformation. This is not the case for Ara-adenine, which remains ineffective as a stimulator of adenylate cyclase.

We have examined the necessity of the high *anti* conformation at the adenosine receptor for lymphocytes and cardiac cells. Further investigation of the diverse effects of adenosine and adenosine analogs will define more accurately the structural and conformational requirements for adenylate cyclase stimulation at these and other cell surfaces. The importance of an understanding of the conformational basis of the biological effects of nucleoside analogs is difficult to overemphasize. The utilization of this information will result in the elimination of unwanted immunosuppressive, cytotoxic, and cardiotoxic effects associated with many nucleoside analogs. The creation of nucleoside analogs that are selectively toxic to the modified metabolism of a target disease or that may selectively modify either cell-mediated or humoral immunity is facilitated by a knowledge of the nucleoside conformations specified by enzyme active sites or cell surface receptors.

The authors thank the National Institutes of Health (Grant GM12862-12) and the University of Utah (Biomedical Research Support Grant FR-07092) for their financial support.

The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

1. Green, H. & Chan, T. (1973) *Science* **182,** 836–837.
2. Ishii, K. & Green, H. (1973) *J. Cell Sci.* **13,** 429–439.
3. Gülen, S., Smith, P. C. & Tremblay, G. C. (1974) *Biochem. Biophys. Res. Commun.* **56,** 934–939.
4. Ito, K. & Uchino, H. (1973) *J. Biol. Chem.* **248,** 4782–4785.
5. Wolberg, G., Zimmerman, T. P., Hiemstra, K., Winston, M. & Chu, L. C. (1975) *Science* **187,** 957–959.
6. Snyder, F. E. & Seegmiller, J. E. (1976) *FEBS Lett.* **66,** 102–106.
7. Planet, G. & Fox, I. H. (1976) *J. Biol. Chem.* **254,** 5839–5844.
8. Bagnara, A. S. & Finch, L. R. (1974) *Eur. J. Biochem.* **41,** 421–430.
9. Becker, M. A. (1976) *Biochim. Biophys. Acta* **435,** 132–144.
10. Skaper, S. D., Willis, R. C. & Seegmiller, J. E. (1976) *Science* **193,** 587–588.
11. Carson, D. A. & Seegmiller, J. E. (1976) *J. Clin. Invest.* **57,** 274–282.
12. Meyskens, F. L. & Williams, H. E. (1971) *Biochim. Biophys. Acta* **240,** 170–179.
13. Parks, R. E., Jr. & Brown, P. R. (1973) *Biochemistry* **12,** 3294–3302.
14. Snyder, F. F., Mendelsohn, J. & Seegmiller, J. E. (1976) *J. Clin. Invest.* **58,** 654–666.
15. Zimmerman, T. P., Rideout, J. L., Wolberg, G., Duncan, G. S. & Elion, G. B. (1976) *J. Biol. Chem.* **251,** 6757–6765.
16. Sattin, A. & Rall, T. W. (1970) *Mol. Pharmacol.* **6,** 13–23.
17. Clark, R. B., Gross, R., Su, Y. F. & Perkins, J. P. (1974) *J. Biol. Chem.* **249,** 5296–5303.
18. Huang, M. & Daly, J. W. (1974) *Life Sci.* **14,** 489–503.
19. Clark, R. B. & Seney, M. N. (1976) *J. Biol. Chem.* **251,** 4239–4246.
20. Sturgill, T. W., Schrier, B. K. & Gilman, A. G. (1975) *J. Cyclic Nucleotide Res.* **1,** 21–30.
21. Haslam, R. J. & Rosson, G. M. (1975) *Mol. Pharmacol.* **11,** 528–544.
22. Blume, A. J. & Foster, C. J. (1975) *J. Biol. Chem.* **250,** 5003–5008.
23. Zenser, T. V. (1975) *Biochim. Biophys. Acta* **404,** 202–213.
24. Haslam, R. J. & Lynham, J. A. (1972) *Life Sci.* **11,** 1143–1154.
25. Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B. & Meuwissen, H. J. (1972) *Lancet* **ii,** 1067–1069.
26. Dissing, J. & Knudsen, B. (1972) *Lancet* **ii,** 1316–1318.
27. Meuwissen, H. J., Pickering, R. J., Pollara, B. & Porter, I. H., eds. (1975) *Combined Immunodeficiency Disease and Adenosine Deaminase Deficiency* (Academic Press, Inc., New York).
28. Scott, C. R., Chen, S-H. & Giblett, E. R. (1974) *J. Clin. Invest.* **53,** 1194–1196.
29. Yount, J. P., Nichols, H. D., Ochs, S. P., Hammar, C., Scott, R., Chen, S-H., Giblett, E. R. & Wedgewood, R. J. (1974) *J. Pediatr.* **84,** 173–177.
30. van der Weyden, M. B., Buckley, R. H. & Kelley, W. N. (1974) *Biochem. Biophys. Res. Commun.* **57,** 590–595.
31. Trotta, P. P., Smithwick, E. M. & Bolis, M. E. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 104–108.
32. Hirschhorn, R., Beratis, N. & Rosen, F. S. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 213–217.
33. Stein, H. H., Somani, P. & Prasad, R. N. (1975) *Ann. N.Y. Acad. Sci.* **255,** 380–389.
34. Entman, M. L. (1974) in *Advances in Cyclic Nucleotide Research,* eds. Greengard, P. & Robison, G. A. (Raven Press, New York), Vol. 4, pp. 163–193.
35. Berne, R. M. (1963) *Am. J. Physiol.* **204,** 317–322.
36. Dobson, J. S., Jr., Rabin, K. & Berne, R. M. (1971) *Circulation Res.* **29,** 375–384.
37. Huang, M. & Drummond, G. I. (1976) *Biochem. Pharmacol.* **25,** 2713–2719.
38. Stafford, A. (1966) *Br. J. Pharmacol. Chemother.* **28,** 218–227.
39. Hopkins, S. V. (1973) *Biochem. Pharmacol.* **22,** 341–348.
40. Marumoto, R., Yoshioka, Y., Mitashita, O., Shima, S., Imai, K. I., Kawazoe, K. & Honjo, M. (1975) *Chem. Pharm. Bull.* **23,** 759–774.
41. Maguire, M. H., Nobbs, D. M., Einstein, R. & Middleton, J. C. (1971) *J. Med. Chem.* **14,** 415–420.
42. Einstein, R., Angus, J. A., Cobbin, L. B. & Maguire, M. H. (1972) *Eur. J. Pharmacol.* **19,** 246–250.

43. Rein, R., Clarke, G. A. & Harris, F. E. (1970) "Quantum aspects of heterocyclic compounds in chemistry and biochemistry," in *Jerusalem Symposia on Quantum Chemistry and Biochemistry* (Israel Academy of Science and Humanities, Jerusalem, Israel or Academic, New York) Vol. 2, pp. 86–117.
44. Watson, D. G., Suton, D. J. & Tollin, P. (1965) *Acta. Crystallogr.* **19,** 111–124.
45. Neidle, S., Kuhlbrandt, W. & Achari, A. (1976) *Acta. Crystallogr. Sect. B* **32,** 1850–1855.
46. Bunick, G. & Voet, D. (1974) *Acta. Crystallogr. Sect. B* **30,** 1651–1660.
47. Prusiner, P. & Sundaralingam, M. (1976) *Acta. Crystallogr. Sect. B* **32,** 419–426.
48. Miles, D. L., Miles, D. W., Redington, P. K. & Eyring, H. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4257–4260.
49. Wolberg, G., Zimmerman, T. P., Duncan, G., Hiemstra, K. & Elion, G. B. (1976) *Fed. Proc.* **35,** 334, Abstr.
50. Naik, S. R., Witkowski, J. T. & Robins, R. K. (1974) *J. Heterocycl. Chem.* **11,** 57–61.
51. Evans, F. E. & Kaplan, N. O. (1976) *J. Biol. Chem.* **251,** 6791–6797.
52. Rodbell, M., Birnbaumer, L., Pohl, S. L. & Krans, H. M. J. (1971) *J. Biol. Chem.* **246,** 1877–1882.
53. Johnson, D. G., Thompson, W. J. & Williams, R. H. (1974) *Biochemistry* **13,** 1920–1924.
54. Rodbell, M., Lin, M. C. & Salomon, Y. (1974) *J. Biol. Chem.* **249,** 59–65.
55. Lefkowitz, R. J. (1974) *J. Biol. Chem.* **249,** 6119–6124.
56. Schramm, M. & Rodbell, M. (1975) *J. Biol. Chem.* **249,** 7630–7636.
57. Salomon, Y., Lin, M. C., Landos, C., Rendell, M. & Rodbell, M. (1975) *J. Biol. Chem.* **250,** 4239–4245.
58. Chien, L. T., Schabel, F. M., Jr. & Alford, C. A., Jr. (1973) in *Selective Inhibitors of Viral Functions*, ed. Carter, W. A. (CRC Press, Cleveland, OH), pp. 227–256.
59. Kurtz, S. M., Fisken, R. A., Kaump, D. H. & Schardein, J. L. (1968) *Antimicrob. Agents Chem. Res.* **8,** 180–189.
60. Zam, Z. S., Centifanto, Y. M. & Kaufman, H. E. (1974) *Interscience Conference Antimicrob. Agents and Chemother, Amer. Sci. Microbiol.* Vol. 4, Abstr. 139.
61. Steele, R. W., Chapa, I. A., Vincent, M. M., Hansen, S. A. & Keeney, R. E. (1975) *Antimicrob. Agents Chemother.* **7,** 203–207.
62. Lerner, A. M. & Bailey, E. J. (1974) *J. Infect. Dis.* **130,** 549–552.
63. Bourne, H. R., Lichtenstein, L. M., Melmon, K. L., Hennly, C. S., Weinstein, Y. & Shearer, G. M. (1974) *Science* **184,** 19–28.
64. Henney, C. S., Bourne, H. R. & Lichtenstein, L. M. (1972) *J. Immunol.* **108,** 1526–1534.
65. Heppner, G. H., Griswold, D. E., Di Lorenzo, J., Poplin, E. A. & Calabresi, P. (1974) *Fed. Proc.* **33,** 1882–1885.
66. Plescia, D. J., Yamamoto, I. & Shimamura, T. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 888–891.
67. Braun, W. (1974) in *Cyclic AMP, Cell Growth and the Immune Response*, eds. Braun, W., Lichtenstein, L. M. & Parker, C. W. (Springer-Verlag, New York), pp. 4–23.
68. Allen, L. B., Huffman, J. H. & Sidwell, R. W. (1973) *Antimicrob. Agents. Chemother.* **3,** 534–535.
69. David, J. R. & David, R. R. (1972) *Prog. Allergy* **16,** 300–449.
70. Kerbel, R. S. & Edinger, D. (1971) *J. Immunol.* **106,** 917–926.
71. Asofsky, R., Cantor, H. & Tigelaar, R. E. (1971) in *Progress in Immunology*, ed. Amos, B. (Academic Press, New York), pp. 369–381.
72. Gershon, R. K. & Liebhaber, S. A. (1972) *J. Exp. Med.* **136,** 112–127.
73. Arima, T. & Fujii H. (1973) *Biochem. Biophys. Res. Commun.* **55,** 410–416.
74. Quigliata, F., Faig, D., Conklyn, M. & Silber, R. (1974) *Cancer Res.* **34,** 3197–3202.
75. Tsuzuki, J. & Newburgh, R. W. (1975) *J. Neurochem.* **25,** 895–896.
76. Gatti, R. A. & Good, R. A. (1971) *Cancer* **28,** 89–98.
77. Penn, I. & Starzl, T. E. (1973) *Transplant. Proc.* **5,** 943–947.
78. Smyth, J. F. & Harrap, K. R. (1975) *Br. J. Cancer* **31,** 544–549.
79. Zimmer, J., Khalifa, A. S. & Lightbody, J. J. (1975) *Cancer Res.* **35,** 68–70.
80. Scholar, E. M. & Calabresi, P. (1973) *Cancer Res.* **33,** 94–103.
81. Loo, T. L., Lu, K. & Gottlieb, J. A. (1973) *Drug. Metab. Dispos.* **1,** 645–652.
82. Gisler, R. H. & Bell, J. P. (1969) *Biochem. Pharmacol.* **18,** 2115–2122.
83. Amer, M. S. (1975) *Life Sci.* **17,** 1021–1038.
84. Salaman, M. H. (1970) *Proc. R. Soc. Med.* **63,** 11–20.
85. Connors, T. A. (1975) *FEBS Lett.* **57,** 223–233.
86. Goldin, A., Wood, H. B., Jr. & Engle, R. R. (1968) *Cancer Chemother. Rep. Part 2.* **1,** 1–272.
87. Krenitsky, T. A., Tuttle, J. V., Koszalka, G. W., Chen, I. S., Beacham, L. M., III, Rideout, J. L. & Elion, G. B. (1976) *J. Biol. Chem.* **251,** 4055–4061.
88. Drach, J. C., Bus, J. S., Schultz, S. K. & Sandberg, J. N. (1974) *Biochem. Pharmacol.* **23,** 2761–2767.