CYCLIC AMP AND DRUG ACTION[1]

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The initial concept that cyclic AMP is an intermediate in the actions of glucagon and epinephrine has evolved rapidly to include an array of biological phenomena so extensive that virtually every facet of pharmacology is now involved. Hundreds of relevant publications have been reviewed recently (1–3), and a monograph on cyclic AMP is forthcoming (4). Valuable accounts of cyclic AMP effects are available pertaining to polypeptide hormones (5), diazoxide (6), skeletal muscle (7), prostaglandins (8), and insulin secretion (9). It is conceivable that the biological effects of materials as diverse as cardiotonic, psychotropic, contraceptive, antibiotic, and diuretic agents will be understood soon in terms of cyclic AMP action. At present our limited insight into the cyclic AMP-related actions of drugs is the result of our imperfect knowledge of cyclic AMP function at the cellular level. Understanding of cyclic AMP-linked drug effects probably will be attained in terms of the mechanisms of action of the cyclic nucleotide and the means by which its intracellular concentration is controlled. Therefore, the enzymes of cyclic AMP metabolism and certain basic cell functions have been selected as topics under which to summarize research published, with few exceptions, subsequent to previous reviews. Included are substantial additions to the evidence which supports the concept formulated by Sutherland and his colleagues of cyclic AMP as a second messenger. A few reports suggest that the momentum for general acceptance of that exceedingly useful thesis is so great that other possible interpretations may be obscured.

ADENYL CYCLASE

Recent studies are in accord with earlier work in showing enzyme activity to be associated with particles, to have moderately alkaline pH optima, and to be stimulated by magnesium and fluoride (10–15). A soluble cyclase from *E. coli* has been purified 100-fold (16). Fluoride inhibits this enzyme, as well as partially purified cyclase from liver (17) and adipose cells (18). The presence of functional sulfhydryl groups may be inferred from the requirement for dithioerythrotol in frog erythrocytes (14). The apparent K_m for ATP is about 1×10^{-4}M in the presence of fluoride in pineal body (11)

[1] Abbreviations used in this review are: cyclic AMP (adenosine 3′,5′-monophosphate); db-cyclic AMP (N^6,2′-O-dibutyryl adenosine 3′,5′-monophosphate); MJ1999 (4-(2-isopropylamino-1-hydroxyethyl)methanesulfonanilide HCl).

and adipose cell homogenates (19). Nucleotides other than ATP can inhibit adenyl cyclase (12, 14, 20).

The extensive list of biological phenomena in which Ca^{++} may be linked in some way with cyclic AMP-mediated events focuses intense interest on the apparent calcium requirement for stimulation of particulate preparations of adenyl cyclase (17, 18, 21). These and other studies (11, 12, 14, 15) agree with earlier work showing an inhibition of activity by excess calcium.

Recent studies have demonstrated clearly that the plasma membrane contains adenyl cyclase. Both biochemical and morphological criteria have been satisfied in the case of liver (17, 22), and frog erythrocytes (14). A highly convincing, if indirect, demonstration of cyclase location is that ACTH diazotized to p-aminobenzoyl cellulose fibers and thereby rendered insoluble stimulated steroid synthesis in adrenal cell cultures (23).

Studies of the subcellular distribution of adenyl cyclase do not appear to exclude the possibility that a fraction of the enzyme is associated with intracellular organelles. For example, cyclase activity of brain was found in all particulate fractions (24), and a nerve ending fraction exhibited half the specific activity of crude mitochondrial starting material (25). Fractions of cell homogenates sedimenting as nuclei, mitochondria, and microsomes contain fragments of plasma membrane, but it is conceivable that cyclase occurs in other intracellular structures in adrenal (21), heart (26), kidney, myometrium, and mammary glands (27).

Hormone-responsive cyclase activity is operationally separate from fluoride-stimulated activity in pineal body (11), frog erythrocytes (14), and ovary (28). Adenyl cyclase of liver responsive to epinephrine can be differentiated from glucagon-sensitive activity (17, 22, 29). Detailed study of adenyl cyclase of adipose cells demonstrated that multiple hormone-specific sites affect the activity of a single adenyl cyclase (30). Thus, adenyl cyclase activity and hormone sensitivity function as distinct entities. The nature of the linkage between them remains a matter of speculation. Fragmentary evidence points to the existence of a soluble activator for adenyl cyclase. Fractions possessing no cyclase activity which augment or preserve that of particles have been noted for rat cerebellum (24), rat liver (29), and BHK cells (31).

New illustrations of adaptive change in the level of adenyl cyclase activity include loss of activity in failing myocardium (32), increased cyclase response to norepinephrine in denervated pineal body (33), and a rise in cyclase of adipose tissue after fasting (34). *De novo* synthesis of adenyl cyclase in adipose tissue of the hyperthyroid rat has been reported (35) as well as stimulation immediately after the addition of thyroid hormones to heart homogenates (36). Hypersensitivity due to adrenergic denervation is not associated with altered cyclase levels in the cat heart (37). Renal cortex cyclase activity nearly doubled after water deprivation (38), and endogenous cyclic AMP was elevated in the cortex to a greater degree than in the

medulla (39). These changes were attributed to the action of endogenous vasopressin released during water deprivation.

Cyclic Nucleotide Phosphodiesterase

Enzymes which catalyze hydrolysis of the 3′-bond of cyclic AMP exhibit more varied characteristics than were suggested by the initial studies of beef heart phosphodiesterase. Degradation of cyclic AMP is under control of three components in mutants of *E. coli* (40). At least two enzymes are responsible for phosphodiesterase activity of brain (41) which is distributed among several fractions (24, 25, 42). The liver enzyme is largely soluble (43), and does not hydrolyze db-cyclic AMP in contrast to the enzyme of adipose tissue (44). Heart phosphodiesterase does not interact with db-cyclic AMP as substrate or inhibitor (45).

Recent findings with methylxanthines underscore the desirability of obtaining evidence for inhibition of phosphodiesterase in various tissues and of considering other actions of these agents. Theophylline at low levels inhibits the phosphodiesterase activity of rat liver (43). High concentrations inhibit phosphodiesterase activity of adipose cell fragments (18, 19), but are much less effective in the case of homogenates of ovary (28), brain (42), and toad bladder epithelium (46). Previous studies have shown that methylxanthines at levels unlikely to inhibit phosphodiesterase appreciably when measured under optimal conditions may nonetheless increase cyclic AMP in tissue. In studies of intact cells in which low concentrations of theophylline potentiated other phenomena such as lipolysis (35, 44), or DNA synthesis (47), it is less certain that phosphodiesterase inhibition can be invoked as the explanation. A series of purine and pyrimidine derivatives inhibit adipose tissue phosphodiesterase, but guanosine and inosine inhibit rather than augment lipolysis (48).

Numerous effects of methylxanthines not attributed to phosphodiesterase inhibition have important implications for cyclic AMP-related research. Catecholamines are released from brain (49) and heart (50) by methylxanthines. Concentrations of theophylline which potentiate phosphorylase activating effects of catecholamines on the heart (presumably cyclic AMP-mediated) depress the myocardium (51). Caffeine exerts effects not mediated by cyclic AMP upon an energy transfer system linked to calcium accumulation in sarcoplasmic reticulum (52). Theophylline inhibits cyclic AMP accumulation in electrically stimulated slices of cerebral cortex, which is contrary to the effect predicted from phosphodiesterase inhibition (53). The increase in chloride permeability caused by theophylline applied to the frog skin was attributed to a locus other than phosphodiesterase (54). The purified adenyl cyclase of frog erythrocytes is inhibited by theophylline (14).

Diazoxide and other benzothiadiazines have been shown previously to inhibit cyclic nucleotide phosphodiesterase, but is it unlike that this property fully accounts for the drug-associated effects of hyperglycemia, hypotension,

tachycardia, and lipolysis (6). It is clear that the hyperglycemic effect results from inhibition of insulin release and peripheral phenomena closely resembling those of catecholamine release. Inhibition of phosphodiesterase activity in islet cells probably enhances rather than restricts the release of insulin. The inhibition of insulin secretion by diazoxide is antagonized by tolbutamide. A related sulfonylurea, chlorpropamide, inhibits the effect of exogenous cyclic AMP in promoting osmotic water flow in the toad bladder (55). Studies on aortic segments revealed a striking competitive blockade by diazoxide of calcium-effected shortening in the presence of norepinephrine (56).

Phosphodiesterase activity in muscle and liver dependent upon insulin and corticosteroids has been described (57). However, others were unable to discern effects of insulin on liver (43) or rat adipose tissue phosphodiesterase (44).

Imidazole stimulates the activity of phosphodiesterase from brain (42), liver (43), and adipose tissue (58) in accord with its known effect on the beef heart enzyme. Imidazole antagonizes the serum calcium response to db-cyclic AMP in parathyroidectomized rats (59) and reduces the lipolytic response of isolated adipose tissue to cyclic AMP and db-cyclic AMP (60). That imidazole stimulates phosphodiesterase and thereby lowers intracellular cyclic AMP is suggested, but the intracellular concentration of imidazole is likely to be very low in comparison to the concentration needed to stimulate the enzyme.

Nicotinic acid did not stimulate phosphodiesterase of fat pad (58, 61) and liver (43). It blocks db-cyclic AMP-induced lipolysis of isolated adipose cells (44), but 5-methylpyrazole-3-carboxylic acid, a compound with many similar actions, does not (58). Interference with cyclic AMP synthesis is a likely mechanism of action for these compounds (34, 58, 61, 62).

Intracellular Cyclic AMP

Cyclic AMP is formed from ATP in the reaction catalyzed by adenyl cyclase, but it may be removed by hydrolysis of the 3′-phosphate bond, by translocation from the intracellular space, and as noted below, by reversal of the adenyl cyclase reaction and perhaps by adenylation of macromolecules. Clearly, the control of the intracellular concentration of cyclic AMP is complex.

Rapid increases following application of an agonist to intact tissue occur in brain (53), heart (63), liver (64), thyroid (65), skeletal muscle (66), adipose cells (67), and uterus (68). Concentrations of cyclic AMP may differ greatly when different agonists are applied to the same cells, and may attain levels in excess of the concentration needed for maximal stimulation of glycogenolysis and gluconeogenesis (64). Typically the new level decays in the continued presence of an agonist. After a two-hour incubation of liver slices with epinephrine, cyclic AMP levels were little different from

controls although cyclic AMP-mediated changes in gluconeogenesis continued (69).

Turnover rather than concentration of cyclic AMP may be expected to correlate with many physiological events. Although such data are not presently available for cyclic AMP, the device of labeling cyclic AMP by pre-labeling intracellular ATP together with the use of one of the established methods for measuring absolute amounts of cyclic AMP should make such measurements feasible. A limitation to be recognized is that multiple pools of cyclic AMP exist in complex tissues, and probably exist within single cell types. About 60 per cent of cyclic AMP in liver was found with particles (64). An hypothesis that separate intracellular pools of cyclic AMP exist in adipose cells has been devised in considering the lack of parallelism between cyclic AMP levels and lipolytic response (67). Potentiation of lipolysis by theophylline in the case of norepinephrine but not of ACTH (70) could be similarly interpreted.

Evidence for the existence from quantitative comparisons using intact tissues and a variety of agonists and antagonists. When an extensive list of species, tissues, experimental conditions, responses, and compounds are considered, it is clear that a large spectrum of receptor types exists. The suggestion that stimulation of β-receptors results in an increase of cyclic AMP and stimulation of α-receptors a decrease, was based upon measurements at one time interval after single doses of epinephrine, propranolol, and phentolamine (71). Demonstrations that cyclic AMP formation in the perfused liver is stimulated equally well by epinephrine and norepinephrine (64), that levo- and dextro-propranolol block insulin secretion (72), and that effects of both norepinephrine and isoproterenol on iodine uptake by thyroid cells are blocked by phentolamine (73) illustrate a few of the problems in applying the concept generally. However, difficulties of characterizing adrenergic receptors, which are in part semantic problems, should not obscure the fact that catecholamines do appear to lower cyclic AMP levels in some instances. Norepinephrine decreases cyclic AMP levels in the frog skin treated with melanocyte stimulating hormone (74), and the toad bladder response to epinephrine and norepinephrine is consistent with a lowering of cyclic AMP (75).

Ambiguities in relating cyclic AMP and adrenergic events may be caused by blocking agents that affect numerous parameters in addition to inhibition at α- and β-receptor sites. The α-blocking agents, phenoxybenzamine and phentolamine, inhibit extraneuronal accumulation of norepinephrine in the heart (76), and dihydroergotamine blocks effects of cyclic AMP on liver (77). Propranolol and other β-adrenergic blockers inhibit lipolysis in response to db-cyclic AMP but at concentrations which probably inhibit adipose cell metabolism generally (78). The capacity of β-adrenergic antagonists to inhibit calcium transport by endoplasmic reticulum (79) has important implications for numerous other phenomena, such as the block by MJ1999 of insulin secretion in response to cyclic AMP (72).

New examples of prostaglandin E_1 effects for which no unifying interpretation at the molecular level is presently available include increased steroidogenesis by rat adrenal (80), increased glucose oxidation and iodine binding by thyroid (81, 82), and stimulation of cyclase in platelets (83, 84). No effect on cyclase of adrenal tumor was noted (12), but it is inferred that in isolated collecting tubules of kidney (85), gastric mucosa (86), and adipose cells (87), prostaglandin E_1 interferes with peptide hormone stimulation of adenyl cyclase.

The mechanism by which cardiac glycosides antagonize cyclic AMP-related lipolysis (19, 88), carbohydrate metabolism (89), and ion permeability (90) has not been defined fully. In adipose cells, a likely locus is an ion transport system, since potassium affects lipolysis (88) and potentiates ACTH stimulation of cyclase activity (18). Excess potassium ion appears to inhibit epinephrine stimulation of heart adenyl cyclase (63) but blocks the action of cyclic AMP on the phosphorylase activating system of muscle (91).

Mechanism of Action of Cyclic AMP

Studies of the structure of cyclic AMP by means of X-ray diffraction reveal two forms with glycosidic bond angles different from that of 5′-AMP (92). Reversibility of the adenyl cyclase reaction and a high energy content of the 3′-phosphate bond of cyclic AMP have been deduced from experiments with soluble adenyl cyclase (93), but attempts to obtain reversal of the cyclase reaction with purified enzyme of frog erythrocyte (14) and the soluble cyclase of *E. coli* (16) have been unsuccessful.

Work on the stimulation of muscle phosphorylase kinase has culminated in the purification of a protein kinase which requires cyclic AMP for activity (94). Subsequently, the occurrence of similar protein kinases dependent upon cyclic AMP has been shown in liver (95), brain (96), *E. coli* (97), and adipose cells (98). The protein kinases effectively phosphorylate histones, which then may result in unmasking of DNA. The induction of enzyme synthesis, noted below, may depend on this phenomenon. Stimulation of glycogen synthesis I kinase by cyclic AMP (99–101) indicates that a protein kinase serves a function analogous to that in the phosphorylase activating system.

Speculation that activation of the protein kinase might occur by adenylation derives from the finding of reversibility of the adenyl cyclase catalyzed reaction and the precedent of adenylation by ATP of glutamine synthetase (96). The evidence which exists fails to support adenylation of enzymes by cyclic AMP, but it is far from conclusive. The interpretation that cyclic AMP does not bind to intact tissue is limited by lack of sensitivity and precision of the methods (46). The inability to detect an adenylated protein in studies of muscle phosphorylase kinase may be caused by the fact that only a minute fraction of the material tested was phosphorylase kinase kinase, the cyclic AMP-sensitive enzyme.

Cyclic AMP has been shown to alter the binding of other ligands to purified phosphofructokinase of skeletal muscle and heart (102). These phenomena may be of minor functional importance for phosphofructokinase, since ADP and AMP exert similar effects. However, the cyclic nucleotide may affect other enzymes by an analogous mechanism. For example, cyclic AMP inhibits the TPN specific malic enzyme of *E. coli* (103). A related concept is that an enzyme may be altered so that its response to a given concentration of cyclic AMP is changed. Such an explanation has been proposed for insulin effects on glycogen synthetase I kinase (99), and for adrenal corticoid effects on gluconeogenesis (64).

Cyclic AMP, db-cyclic AMP, and other analogues, when applied to intact cells, may exert actions not related to the cyclic phosphate moiety. Several other adenine nucleotides duplicate the effects of cyclic AMP applied to adrenal cells in tissue culture (104), and to intestinal smooth muscle (105). Numerous effects of noncyclic adenine nucleotides on cardiovascular parameters have been recorded. However, the possibility is not excluded that intracellular cyclic AMP may play an important role in some instances where the adenine nucleotides mimic the effects of exogenous cyclic AMP. Concentrations of adenosine and adenine mononucleotides as low as 10μM caused large accumulations of cyclic AMP in slices of cerebral cortex (53). Although this phenomenon was not duplicated in bovine thyroid slices (65), attempts to confirm or deny its occurrence in other tissues would seem essential.

The use of nucleotide analogues to elucidate the mechanism of action of cyclic AMP has been only partially exploited. A receptor site for cyclic AMP mediation of liver glycogenolysis is also affected by the 3′,5′-monophosphates of inosine and tubericidin, but only minimally by comparable analogues of guanosine, thymidine, and uridine, and not at all by that of cytidine (106). The protein kinase of adipose cells is also stimulated by inosine 3′,5′-monophosphate (98). The response to cyclic AMP of pancreas is inhibited by 3′-AMP (107), and of adipose cells by guanosine 3′,5′-monophosphate (108). Induction of phosphopyruvate carboxylase in liver by cyclic AMP is not duplicated by other 2′,3′- and 3′,5′-mononucleotides (109). Thyrocalcitonin blocks both db-cyclic AMP and parathyroid hormone effects on calcium and phosphate metabolism (110).

Two unifying concepts for phenomena which involve membranes, cyclic AMP, and calcium ion have been proposed (111). In the first hypothesis, cyclic AMP is viewed as a relatively stable compound, not participating in other cellular reactions. Its important attribute is that it does not chelate calcium, whereas membrane-bound ATP does. Calcium ion release is visualized as a consequence of the stimulation of adenyl cyclase resulting in the conversion of membrane-bound ATP to cyclic AMP. As noted above, it has subsequently been learned that reversal of the adenyl cyclase catalyzed reaction can occur. Elevating cyclic AMP by means of phosphodiesterase inhibition should generate membrane-bound ATP, increase calcium chelating

capacity, and inhibit those events now recognized as cyclic AMP mediated. A more direct criticism comes from the finding that cyclic AMP itself releases calcium ion from the perfused liver (112). In the second hypothesis, cyclic AMP is regarded as a reactive compound which increases permeability of cell membranes to calcium ion. Numerous phenomena are compatible with that idea, including the recent examples of an apparent requirement for calcium ion in the cyclic AMP-linked metabolic response of heart (63), thyroid (113), and kidney (114). It is conceivable that parathyroid stimulated bone resorption is due to cyclic AMP-mediated calcium transport (15).

Integrated Cellular Events

Carbohydrate metabolism.—Greater understanding of the linkage between adrenergic stimulation and glycogenolysis has been attained. In the calcium-deficient rat heart, epinephrine-induced activation of phosphorylase is diminished in spite of a rise in cyclic AMP and phosphorylase kinase activation (63). Some dissociation of cyclic AMP levels from phosphorylase activation occurs in normal mice as well as in those lacking phosphorylase kinase (66).

Evidence for cyclic AMP effects on mitochondrial metabolism has been obtained in studies of metabolites in renal cells after parathyroid hormone treatment (114), and in studies of oxidative phosphorylation in brown fat cells (115). A mitochondrial locus, possibly pyruvate carboxylase, is favored as the site of cyclic AMP action in gluconeogenesis in liver (116, 117).

The role of a cyclic AMP-activated triglyceride lipase augmenting gluconeogenesis remains controversial. Glucagon and cyclic AMP stimulate the release of free fatty acids and increase acyl coenzyme A esters in liver slices (118). Effects of oleate and glucagon on several metabolic parameters of perfused liver have been interpreted as supporting the concept that cyclic AMP activates a lipase and that fatty acid oxidation contributes to gluconeogenesis (119). Interpretations from closely analogous studies of perfused liver differ (116, 117). There is agreement that adrenal corticoids affect gluconeogenesis through mechanisms other than increased cyclic AMP concentration in the liver (64, 69, 120). Insulin lowers cyclic AMP levels in liver following epinephrine or glucagon (64), but fails to do so in diaphragm (99, 121). The conversion of glycogen synthetase from the D to the I form is accomplished, however. Findings that db-cyclic AMP depresses several metabolic parameters in diaphragm led to the suggestion that insulin may promote the synthesis of a cyclic AMP antagonist (122).

Lipolysis.—Cyclic AMP effects on lipolysis appear to be separable from those on carbohydrate utilization by adipose cells (87). The lipolytic response subsequent to cyclic AMP formation is under complex control, but the newly discovered protein kinase of adipose cells may play a pivotal role by phosphorylating lipase (98). Sulfhydryl groups are essential for activation of lipase (123), and inosine, adenosine, and deoxyadenosine may be in-

hibitory (48). Insulin appears to block adenyl cyclase while polyene antibiotics and bacterial protease, which mimic effects of insulin on lipolysis, do so by different mechanisms (67). The striking disparities between relative concentrations of intracellular cyclic AMP and lipolysis obtained with these agents and with several other antibiotics (124) are not easily reconciled with the second messenger concept. Failure of insulin to lower adipose cell cyclic AMP (62) also argues that a unified explanation relating insulin, cyclic AMP, and lipolysis has not yet been devised.

The complex response of plasma free fatty acids to cyclic AMP administration *in vivo* reflects actions on many cell types in addition to adipose tissue. In the human a prompt fall in free fatty acids is followed by an increased level (125). The release of insulin in response to db-cyclic AMP infusion in rats may account for the fall in plasma glycerol (126).

Contractility.—In the heart, a close parallelism of cyclic AMP levels and inotropic effects of adrenergic agents has been established (1). Glucagon acting at a locus separate from the catecholamine site effectively stimulates both the inotropic response and adenyl cyclase of heart (127, 128). The suggestion that the inotropic response is dissociated from cyclic AMP changes after glucagon is based upon measurements of tissue which may not have been sufficiently rapidly frozen and extracted (129, 130).

The possibility exists that cyclic AMP is involved in cytoplasmic motility coupled to secretion and translocation of cytoplasmic particles. A graphic illustration of the capacity of cyclic AMP to exert such effects is the reversible dispersion of granules in melanocytes (74). Indirect evidence for histamine release from leukocytes in a process which involves microtubules and cyclic AMP has been obtained (131, 132). A related speculation links cyclic AMP, microtubules, and the intracellular movement of insulin granules in the β-cell (133).

Secretion.—Cyclic AMP has been implicated recently in renin elaboration by renal cells (134) and exocrine secretion by pancreas (107). Insulin is secreted from β-cells in response to secretin, pancreozymin, and gastrin (135) as well as to glucagon and isoproterenol. Phentolamine pre-treatment augments the insulin secretory response not only to epinephrine (136) but also to tranylcypromine (137). Inhibition by β-adrenergic blocking agents occurs with tranylcypromine, prostaglandin E_1, and cyclic AMP-induced secretion (72, 137).

Evidence has been obtained that cyclic AMP participates in the release from the anterior pituitary of ACTH (138), FSH (139), and TSH (140). Growth hormone release may depend upon cyclic AMP, but the effects of epinephrine on that process differ from those on insulin secretion (136, 141).

Cyclic AMP has been implicated in TSH action on thyroid, but the events associated with thyroxin secretion are not readily distinguished from coincidental changes in carbohydrate and phospholipid metabolism. Thyroid stimulating hormone increases cyclic AMP accumulation (65). Iodine bind-

ing and colloid formation are increased by TSH, db-cyclic AMP, and cyclic AMP (82, 142), and the effects are potentiated by methylxanthines. Iodine binding is also increased by epinephrine, but theophylline is without effect (73). TSH and db-cyclic AMP stimulate glucose oxidation and ^{32}P incorporation into phospholipids (82) through a metabolic sequence requiring calcium ion (113). Disparities in the pattern linking cyclic AMP with these metabolic events are that db-cyclic AMP and theophylline fail to potentiate effective doses of TSH (143) and that increased labeling of phospholipids may be due to greater uptake of ^{32}P (144). Detailed study of bovine thyroid slices indicated that cyclic AMP is not involved in the TSH-stimulated oxidation of glucose-1-^{14}C and pyruvate-^{14}C (145). The interpretation that cyclic AMP does not mediate biosynthetic responses to TSH (144) was based upon ^{32}P and ^{125}I incorporation into macromolecules and therefore is not necessarily in conflict with the finding that cyclic AMP accelerates protein synthesis by thyroid polyribosomes (146).

Questions of specificity and site of cyclic AMP stimulation of adrenal steroidogenesis are not resolved. Additional evidence that increased capacity for 11β-hydroxylation by stimulated cells in culture is a mitochondrial function has been obtained (104). The stimulation of corticosteroid synthesis by prostaglandins, like that of cyclic AMP, is blocked by inhibiting protein synthesis (80). Multiple sites for cyclic AMP action on the adrenal are suggested by the capacity of db-cyclic AMP to sustain adrenal content of DNA, RNA, and protein of hypophysectomized rats (147). An important reservation in interpreting cyclic AMP effects on steroidogenesis derives from the finding that pregnenolone accumulation in response to cyclic AMP is due to inhibition of a subsequent step (148).

Neurohumoral transmission.—Electrical impulses applied to slices of cerebral cortex increased the content of cyclic AMP (53). The effect was enhanced by norepinephrine and histamine, but in contrast to previous findings, the amine effects were inhibited by theophylline. This fact and other disparities in comparison to studies using slices prepared at 4° C complicate possible interpretations, but it appears that cyclic AMP accumulation cannot be attributed to release of endogenous histamine or norepinephrine. Also, regional distributions of adenyl cyclase and phosphodiesterase do not correlate with catecholamine content (24, 42) and catecholamines fail to stimulate adenyl cyclase from certain brain areas. Recently, direct evidence for cyclic AMP function in the nervous system has been obtained by injecting or implanting nucleotides into various brain regions (149, 150), and by perfusing the ventricles (151). Hyperactivity, hyperthermia, mydriasis, salivation, pilo-erection, and convulsions were observed at short time intervals (149, 151), and hyperphagia and prolongation of the estrous cycle over longer periods (150). The mechanism of these diverse effects at the neuronal level is not known, but the earlier inference of enhanced release of acetylcholine at motor nerve endings (152) is supported by the increased frequency of miniature end plate potentials in response to db-cyclic AMP and

theophylline (153). An analogous mechanism may participate in the release of neurotransmitters and hypophyseal releasing factors (111, 150).

Permeability.—The heterogeneity of cell types in the amphibian bladder may be responsible for recent qualifications in interpreting the role of cyclic AMP in sodium and water permeability. Smooth muscle cells of the toad bladder contribute to changes in cyclic AMP content in response to adrenergic agents and to changes in surface area of the bladder under study (75). Isolated epithelium of frog bladder exhibits a greatly reduced response to exogenous cyclic AMP and theophylline (154). A potentially valuable tool in pursuing cyclic AMP effects on ion transport is the duck erythrocyte which accumulates K^+ and water in response to db-cyclic AMP and β-adrenergic agonists (155). Potassium ion is reported to enter (77) and to leave (64, 112) the perfused liver in response to cyclic AMP.

Indications that cyclic AMP may mediate effects of aldosterone on toad bladder (156) and estrogen on amino acid uptake by uterus (157) are of interest in view of previous unsuccessful attempts to link steroid and cyclic nucleotide effects.

Protein synthesis.—Synthesis of tyrosine aminotransferase, glucose-6-phosphatase, and phosphopyruvate carboxykinase is induced in fetal rat liver explants by cyclic AMP or db-cyclic AMP by a mechanism separate from that of hydrocortisone stimulation (109, 158, 159). Formation of serine dehydratase of rat liver in addition to these enzymes is stimulated by glucagon and epinephrine as well as by cyclic AMP (160). Clear dissociation of corticosteroid induction of tyrosine aminotransferase from cyclic AMP occurs in rat hepatoma (161). The synthesis of several lysosomal enzymes by bone explants is stimulated by db-cyclic AMP (162).

A single locus for all cyclic AMP actions on the protein synthesizing apparatus is unlikely in view of the fact that effects ranging from increased DNA synthesis (47) to stimulation of polyribosomes (146, 163) have been observed. Cyclic AMP increases synthesis of β-galactosidase in *E. coli* and antagonizes glucose repression. The use of mutant strains led to the conclusion that cyclic AMP acts at the level of transcription of DNA at the *lac* promoter site. The suggestion that cyclic AMP functions at the level of glucose conversion to glucose-6-phosphate rather than at the level of DNA transcription is not supported by studies which show a cyclic AMP effect on an early stage of transcription (164).

Methods

Adenyl cyclase is conveniently measured by the use of labeled ATP combined with chromatographic separation of cyclic AMP (10, 19, 20, 27). A barium-zinc precipitation of interfering materials is an important contribution (10), but limitations remain on the separation of the cyclic nucleotide (19). Although methylxanthines may eliminate phosphodiesterase activity in some instances (127), cyclic AMP can be rapidly degraded (28). The addi-

tion of nonradioactive cyclic AMP and of an ATP-regenerating system is helpful (18, 20).

New procedures for cyclic nucleotide phosphodiesterase measurement include continuous monitoring by means of a coupled enzymatic system (57), and a titrimetric assay (165). A micromethod applicable to samples of frozen dried tissue weighing one μg is available (42). Procedures in which the net conversion of cyclic AMP to 5′-AMP by tissue extracts is assessed are subject to the reservation that cyclic AMP may be converted to 5′-AMP by a series of reactions rather than by hydrolytic cleavage (40).

For measurements of endogenous cyclic AMP, methods based upon the hydrolysis of cyclic AMP to 5′-AMP by means of purified phosphodiesterase followed by the conversion to ATP offer certain advantages. High sensitivity is attained by enzymatically amplifying the ATP and using a fluorometric procedure (120), or ^{32}P can be enzymatically incorporated into ATP (166). Operational simplicity of the enzymatic radioisotopic displacement method (41) is a clear advantage for many applications.

In contrast to measures of absolute amount of cyclic AMP, relative changes can be estimated by labeling intracellular ATP (67, 129, 167). Simplicity of these procedures is balanced by the fact that the assumption of constant specific activity of ATP may not be valid. Changes in turnover of adenine nucleotides have been noted in related experiments (168). In measurements of cyclic AMP after adding labeled ATP to the medium (62), two findings suggest that adenyl cyclase in the intact cells is not freely accessible to ATP; the apparent K_m for ATP is high in comparison to that of broken cell preparations (19) and activity increases with hypo-osmolarity.

All methods for measurement of endogenous cyclic AMP depend for validity upon rapid freezing of tissue because major changes occur within seconds in many cell types.

LITERATURE CITED

1. Sutherland, E. W., Robison, G. A., Butcher, R. W., *Circulation,* **37,** 279–306 (1968)
2. Butcher, R. W., Robison, G. A., Hardman, J. G., Sutherland, E. W., *Advan. Enzyme Reg.,* **6,** 357–89 (1968)
3. Robison, G. A., Butcher, R. W., Sutherland, E. W., *Ann. Rev. Biochem.,* **37,** 149–74 (1968)
4. Robison, G. A., Butcher, R. W., Sutherland, E. W., *Cyclic AMP* (Academic Press, New York, in press)
5. Margoulies, M., Ed., *Protein and Polypeptide Hormones, Part I* (Excerpta Medica Foundation, Amsterdam, 325 pp., 1968)
6. Smith, H. M., Ed., *Ann. N.Y. Acad. Sci.,* **150,** 191–467 (1968)
7. Bowman, W. C., Nott, M. W., *Pharmacol. Rev.,* **21,** 27–72 (1969)
8. Horton, E. W., *Physiol. Rev.,* **49,** 122–61 (1969)
9. Frohman, L. A., *Ann. Rev. Physiol.,* **31,** 353–82 (1969)
10. Krishna, G., Weiss, B., Brodie, B. B., *J. Pharmacol. Exptl. Therap.,* **163,** 379–85 (1968)
11. Weiss, B., *J. Pharmacol. Exptl. Therap.,* **166,** 330–38 (1969)
12. Taunton, O. D., Roth, J., Pastan, I., *J. Biol. Chem.,* **244,** 247–53 (1969)
13. Murad, F., Strauch, B. S., Vaughan, M., *Biochim. Biophys. Acta,* **177,** 591–98 (1969)
14. Rosen, O. M., Rosen, S. M., *Arch. Biochem. Biophys.,* **131,** 449–56 (1969)
15. Chase, L. R., Fedak, S. A., Aurbach, G. D., *Endocrinology,* **84,** 761–68 (1969)
16. Tao, M., Lipmann, F., *Proc. Natl. Acad. Sci. U.S.,* **63,** 86–92 (1969)
17. Marinetti, G. V., Ray, T. K., Tomasi, V., *Biochem. Biophys. Res. Commun.,* **36,** 185–93 (1969)
18. Birnbaumer, L., Pohl, S. L., Rodbell, M., *J. Biol. Chem.,* **244,** 3468–76 (1969)
19. Bar, H.-P., Hechter, O., *Anal. Biochem.,* **29,** 476–89 (1969)
20. Cryer, P. E., Jarett, L., Kipnis, D. M., *Biochim. Biophys. Acta,* **177,** 586–90 (1969)
21. Bar, H.-P., Hechter, O., *Biochem. Biophys. Res. Commun.,* **35,** 681–86 (1969)
22. Pohl, S. L., Birnbaumer, L., Rodbell, M., *Science,* **164,** 566–67 (1969)
23. Schimmer, B. P., Ueda, K., Sato, G. H., *Biochem. Biophys. Res. Commun.,* **32,** 806–10 (1968)
24. Weiss, B., Costa, E., *Biochem. Pharmacol.,* **17,** 2107–16 (1968)
25. De Robertis, E., Arnaiz, G. R. D. L., Alberici, M., Butcher, R. W., Sutherland, E. W., *J. Biol. Chem.,* **242,** 3487–93 (1967)
26. Entman, M. L., Levey, G. S., Epstein, S. E., *Biochem. Biophys. Res. Commun.,* **35,** 728–33 (1969)
27. Dousa, T., Rychlik, I., *Life Sci.,* **7,** 1039–44 (1968)
28. Dorrington, J. H., Baggett, B., *Endocrinology,* **84,** 989–96 (1969)
29. Bitensky, M. W., Russell, V., Robertson, W., *Biochem. Biophys. Res. Commun.,* **31,** 706–12 (1968)
30. Birnbaumer, L., Rodbell, M., *J. Biol. Chem.,* **244,** 3477–82 (1969)
31. Burk, R. R., *Nature,* **219,** 1272–75 (1968)
32. Sobel, B. E., Henry, P. D., Robison, A., Bloor, C., Ross, J., Jr., *Circulation Res.,* **24,** 507–12 (1969)
33. Weiss, B., *J. Pharmacol. Exptl. Therap.,* **168,** 146–52 (1969)
34. Brodie, B. B., Krishna, G., Hynie, S., *Biochem. Pharmacol.,* **18,** 1129–34 (1969)
35. Krishna, G., Hynie, S., Brodie, B. B., *Proc. Natl. Acad. Sci. U.S.,* **59,** 884–89 (1968)
36. Levey, G. S., Epstein, S. E., *Biochem. Biophys. Res. Commun.,* **33,** 990–95 (1968)
37. Sobel, B. E., Dempsey, P. J., Cooper, T., *Biochem. Biophys. Res. Commun.,* **33,** 758–62 (1968)
38. Schultz, G., *Naunyn-Schmiedebergs Arch. Exptl. Pathol. Pharmakol.,* **263,** 250–51 (1969)
39. Senft, G., Hoffmann, M., Munske, K., Schultz, G., *Pfluegers Arch. Ges. Physiol.,* **298,** 348–58 (1968)
40. Monard, D., Janecek, J., Rickenberg, H. V., *Biochem. Biophys. Res. Commun.,* **35,** 584–91 (1969)
41. Brooker, G., Thomas, L. J., Jr., Appelman, M. M., *Biochemistry,* **7,** 4177–81 (1968)
42. Breckenridge, B. McL., Johnston, R. E., *J. Histochem. Cytochem.,* **17,** 505–11 (1969)
43. Menahan, L. A., Hepp, K. D., Wieland, O., *European J. Biochem.,* **8,** 435–43 (1969)

44. Blecher, M., Merlino, N. S., Ro'Ane, J. T., *J. Biol. Chem.*, **243,** 3973–77 (1968)
45. Moore, P. F., Iorio, L. C., McManus, M. J., *J. Pharm. Pharmacol.*, **20,** 368–72 (1968)
46. Gulyassy, P. F., *J. Clin. Invest.*, **47,** 2458–68 (1968)
47. Malamud, D., *Biochem. Biophys. Res. Commun.*, **35,** 754–58 (1969)
48. Davies, J. I., *Nature*, **218,** 349–52 (1968)
49. Berkowitz, B. A., Tarver, J. H., Spector, S., *Federation Proc.*, **28,** 415 (1969)
50. Westfall, D. P., Fleming, W. W., *J. Pharmacol. Exptl. Therap.*, **159,** 98–106 (1968)
51. McNeill, J. H., Nassar, M., Brody, T. M., *J. Pharmacol. Exptl. Therap.*, **165,** 234–41 (1969)
52. Weber, A., *J. Gen. Physiol.*, **52,** 760–72 (1968)
53. Kakiuchi, S., Rall, T. W., McIlwain, H., *J. Neurochem.*, **16,** 485–91 (1969)
54. Cuthbert, A. W., Painter, E., *J. Physiol. (London)*, **199,** 593–612 (1968)
55. Mendoza, S. A., *Endocrinology*, **84,** 411–14 (1969)
56. Wohl, A. J., Hausler, L. M., Roth, F. E., *Life Sci.*, **7,** 381–87 (1968)
57. Senft, G., Schultz, G., Munske, K., Hoffmann, M., *Diabetologia*, **4,** 330–35 (1968)
58. Kupiecki, F. P., Marshall, N. B., *J. Pharmacol. Exptl. Therap.*, **160,** 166–70 (1968)
59. Wells, H., Lloyd, W., *Endocrinology*, **84,** 861–67 (1969)
60. Goodman, H. M., *Proc. Soc. Exptl. Biol. Med.*, **130,** 97–100 (1969)
61. Peterson, M. J., Hillman, C. C., Ashmore, J., *Mol. Pharmacol.*, **4,** 1–9 (1968)
62. Williams, R. H., Walsh, S. A., Hepp, D. K., Ensinck, J. W., *Metabolism*, **17,** 653–68 (1968)
63. Namm, D. H., Mayer, S. E., Maltbie, M., *Mol. Pharmacol.*, **4,** 522–30 (1968)
64. Exton, J. H., Park, C. R., *Advan. Enzyme Reg.*, **6,** 391–407 (1968)
65. Gilman, A. G., Rall, T. W., *J. Biol. Chem.*, **243,** 5867–71 (1968)
66. Lyon, J. B., Jr., Mayer, S. E., *Biochem. Biophys. Res. Commun.*, **34,** 459–64 (1969)
67. Kuo, J. F., De Renzo, E. C., *J. Biol. Chem.*, **244,** 2252–60 (1969)
68. Szego, C. M., Davis, J. S., *Proc. Natl. Acad. Sci. U.S.*, **58,** 1711–18 (1967)
69. Rinard, G. A., Okuno, G., Haynes, R. C., Jr., *Endocrinology*, **84,** 622–31 (1969)
70. Poyart, C. F., Nahas, G. G., *Mol. Pharmacol.*, **4,** 389–401 (1968)
71. Turtle, J. R., Kipnis, D. M., *Biochem. Biophys. Res. Commun.*, **28,** 797–802 (1967)
72. Bressler, R., Vargas-Cordon, M., Brendel, K., *Arch. Internal Med.*, **123,** 248–51 (1969)
73. Maayan, M. L., Ingbar, S. H., *Science*, **162,** 124–25 (1968)
74. Abe, K., Butcher, R. W., Nicholson, W. E., Baird, C. E., Liddle, R. A., Liddle, G. W., *Endocrinology*, **84,** 362–68 (1969)
75. Handler, J. S., Bensinger, R., Orloff, J., *Am. J. Physiol.*, **215,** 1024–31 (1968)
76. Eisenfeld, A. J., Axelrod, J., Krakoff, L., *J. Pharmacol. Exptl. Therap.*, **156,** 107–13 (1967)
77. Northrop, G., *J. Pharmacol. Exptl. Therap.*, **159,** 22–28 (1968)
78. Allen, D. O., Ashmore, J., *Biochem. Pharmacol.*, **18,** 1347–54 (1969)
79. Scales, B., McIntosh, D. A. D., *J. Pharmacol. Exptl. Therap.*, **160,** 261–67 (1968)
80. Flack, J. D., Jessup, R., Ramwell, P. W., *Science*, **163,** 691–92 (1969)
81. Kaneko, T., Zor, U., Field, J. B., *Science*, **163,** 1062–63 (1969)
82. Rodesch, F., Neve, P., Willems, C., Dumont, J. E., *European J. Biochem.*, **8,** 26–32 (1969)
83. Wolfe, S. M., Shulman, N. R., *Biochem. Biophys. Res. Commun.*, **35,** 265–72 (1969)
84. Zieve, P. D., Greenough, W. B., III, *Biochem. Biophys. Res. Commun.*, **35,** 462–66 (1969)
85. Grantham, J. J., Orloff, J., *J. Clin. Invest.*, **47,** 1154–61 (1968)
86. Way, L., Durbin, R. P., *Nature*, **221,** 874–75 (1969)
87. Blecher, M., Merlino, N. S., Ro'Ane, J. T., Flynn, P. D., *J. Biol. Chem.*, **244,** 3423–29 (1969)
88. Fain, J. N., *Mol. Pharmacol.*, **4,** 349–57 (1968)
89. Kypson, J., Triner, L., Nahas, G. G., *J. Pharmacol. Exptl. Therap.*, **164,** 22–30 (1968)
90. Field, M., Plotkin, G. R., Silen, W., *Nature*, **217,** 469–71 (1968)

91. Lundholm, L., Mohme-Lundholm, E., Vamos, N., *Acta Physiol. Scand.*, **75,** 187–98 (1969)
92. Watenpaugh, K., Dow, J., Jensen, L. H., Furberg, S., *Science,* **159,** 206–7 (1968)
93. Greengard, P., Hayaishi, O., Colowick, S. P., *Federation Proc.,* **28,** 467 (1969)
94. Walsh, D. A., Perkins, J. P., Krebs, E. G., *J. Biol. Chem.,* **243,** 3763–65 (1968)
95. Langan, T. A., *Science,* **162,** 579–80 (1968)
96. Miyamoto, E., Kuo, J. F., Greengard, P., *Science,* **165,** 63–65 (1969)
97. Kuo, J. F., Greengard, P., *J. Biol. Chem.,* **244,** 3417–19 (1969)
98. Corbin, J. D., Krebs, E. G., *Biochem. Biophys. Res. Commun.,* **36,** 328–36 (1969)
99. Larner, J., Villar-Palasi, C., Goldberg, N. D., Bishop, J. S., Huijing, F., Wenger, J. I., Sasko, H., Brown, N. B., *Advan. Enzyme Reg.,* **6,** 409–23 (1968)
100. Bishop, J. S., Larner, J., *Biochim. Biophys. Acta,* **171,** 374–77 (1969)
101. Goldberg, N. D., O'Toole, A. G., *J. Biol. Chem.,* **244,** 3053–61 (1969)
102. Mansour, T. E., Ahlfors, C. E., *J. Biol. Chem.,* **243,** 2523–33 (1968)
103. Sanwal, B. D., Smando, R., *Biochem. Biophys. Res. Commun.,* **35,** 486–91 (1969)
104. Kowal, J., *Biochemistry,* **8,** 1821–31 (1969)
105. Kim, T. S., Shulman, J., Levine, R. A., *J. Pharmacol. Exptl. Therap.,* **163,** 36–42 (1968)
106. Levine, R. A., *Federation Proc.,* **28,** 707 (1969)
107. Kulka, R. G., Sternlicht, E., *Proc. Natl. Acad. Sci. U.S.,* **61,** 1123–28 (1968)
108. Manganiello, V., Murad, F., Vaughan, M., *Federation Proc.,* **28,** 876 (1969)
109. Yeung, D., Oliver, I. T., *Biochemistry,* **7,** 3231–39 (1968)
110. Rasmussen, H., Pechet, M., Fast, D., *J. Clin. Invest.,* **47,** 1843–50 (1968)
111. Rasmussen, H., Tenenhouse, A., *Proc. Natl. Acad. Sci. U.S.,* **59,** 1364–70 (1968)
112. Friedmann, N., Park, C. R., *Proc. Natl. Acad. Sci. U. S.,* **61,** 504–8 (1968)
113. Zor, U., Lowe, I. P., Bloom, G., Field, J. B., *Biochem. Biophys. Res. Commun.,* **33,** 649–58 (1968)
114. Nagata, N., Rasmussen, H., *Biochemistry,* **7,** 3728–33 (1968)
115. Reed, N., Fain, J. N., *J. Biol. Chem.,* **243,** 2843–48 (1968)
116. Exton, J. H., Park, C. R., *J. Biol. Chem.,* **243,** 4189–96 (1968)
117. Exton, J. H., Park, C. R., *J. Biol. Chem.,* **244,** 1424–33 (1969)
118. Claycomb, W. C., Kilsheimer, G. S., *Endocrinology,* **84,** 1179–83 (1969)
119. Williamson, J. R., Browning, E. T., Thurman, R. G., Scholz, R., *J. Biol. Chem.,* **244,** 5055–64 (1969)
120. Goldberg, N. D., Larner, J., Sasko, H., O'Toole, A. G., *Anal. Biochem.,* **28,** 523–44 (1969)
121. Craig, J. W., Rall, T. W., Larner, J., *Biochim Biophys. Acta,* **177,** 213–19 (1969)
122. Chambaut, A., Eboue-Bonis, D., Hanoune, J., Clauser, H., *Biochem. Biophys. Res. Commun.,* **34,** 283–90 (1969)
123. Marsh, D. G., George, J. M., *J. Biol. Chem.,* **244,** 1381–82 (1969)
124. Kuo, J. F., *Biochem. Pharmacol.,* **18,** 757–66 (1969)
125. Levine, R. A., *Metabolism,* **17,** 34–45 (1968)
126. Bieck, P., Stock, K., Westermann, E., *Naunyn-Schmiedebergs Arch. Exptl. Pathol. Pharmakol.,* **263,** 387–405 (1969)
127. Murad, F., Vaughan, M., *Biochem. Pharmacol.,* **18,** 1053–59 (1969)
128. Levey, G. S., Epstein, S. E., *Circulation Res.,* **24,** 151–56 (1969)
129. La Raia, P. J., Reddy, W. J., *Biochim. Biophys. Acta,* **177,** 189–95 (1969)
130. La Raia, P. J., Craig, R. J., Reddy, W. J., *Am. J. Physiol.,* **215,** 968–70 (1968)
131. Levy, D. A., Carlton, J. A., *Proc. Soc. Exptl. Biol. Med.,* **130,** 1333–36 (1969)
132. Lichtenstein, L. M., Margolis, S., *Science,* **161,** 902–3 (1968)
133. Lacy, P. E., Howell, S. L., Young, D. A., Fink, C. J., *Nature,* **219,** 1177–79 (1968)
134. Michelakis, A. M., Caudle, J., Liddle, G. W., *Proc. Soc. Exptl. Biol. Med.,* **130,** 748–53 (1969)
135. Allan, W., Tepperman, H. M., *Life Sci.,* **8,** 307–17 (1969)
136. Hertelendy, F., Machlin, L., Kipnis, D. M., *Endocrinology,* **84,** 192–9 (1969)
137. Bressler, R., Vargas-Cordon, M.,

Lebovitz, H. E., *Diabetes,* **17,** 617–24 (1968)
138. Fleischer, N., Donald, R. A., Butcher, R. W., *Clin. Res.,* **17,** 23 (1969)
139. Jutisz, M., Paloma de la Llosa, M., *Compt. Rend. Acad. Sci. Paris, Ser. D,* **268,** 1636–39 (1969)
140. Wilber, J. F., Peake, G. T., Utiger, R. D., *Endocrinology,* **84,** 758–60 (1969)
141. Blackard, W. G., Heidingsfelder, S. A., *J. Clin. Invest.,* **47,** 1407–14 (1968)
142. Ensor, J. M., Munro, D. S., *J. Endocrinol.,* **43,** 477–85 (1969)
143. Burke, G., *Endocrinology,* **84,** 1055–62 (1969)
144. Kerkof, P. R., Tata, J. R., *Biochem. J.,* **112,** 729–39 (1969)
145. Gilman, A. G., Rall, T. W., *J. Biol. Chem.,* **243,** 5872–81 (1968)
146. Lissitzky, S., Mante, S., Attali, J.-C., Cartouzou, G., *Biochem. Biophys. Res. Commun.,* **35,** 437–43 (1969)
147. Ney, R. L., *Endocrinology,* **84,** 168–70 (1969)
148. Koritz, S. B., Yun, J., Ferguson, J. J., Jr., *Endocrinology,* **82,** 620–22 (1968)
149. Krishna, G., Ditzion, B. R., Gessa, G. L., *Proc. Intern. Union Physiol. Sci.,* **7,** 247 (1968)
150. Breckenridge, B. McL., Lisk, R. D., *Proc. Soc. Exptl. Biol. Med.,* **131,** 934-35 (1969)
151. McKean, C. M., Peterson, N. A., Raghupathy, E., *Federation Proc.,* **28,** 776 (1969)
152. Breckenridge, B. McL., Burn, J. H., Matschinsky, F. M., *Proc. Natl. Acad. Sci. U.S.,* **57,** 1893–97 (1967)
153. Goldberg, A. L., Singer, J. J., Henneman, E., *Federation Proc.,* **28,** 457 (1969)
154. Parisi, M., Ripoche, P., Bourguet, J., *Pfluegers Arch. Ges. Physiol.,* **309,** 59–69 (1969)
155. Riddick, D. H., Kregenow, F., Orloff, J., *Federation Proc.,* **28,** 339 (1969)
156. Sharp, G. W. G., Kirchberger, M. A., Martin, D. G., Leaf, A., *J. Clin. Invest.,* **47,** 89–90a (1968)
157. Griffin, D. M., Szego, C. M., *Life Sci.,* **7,** 1017–23 (1968)
158. Wicks, W. D., *J. Biol. Chem.,* **244,** 3941–50 (1969)
159. Greengard, O., *Science,* **163,** 891–95 (1969)
160. Jost, J.-P., Hsie, A. W., Rickenberg, H. V., *Biochem. Biophys. Res. Commun.,* **34,** 748–54 (1969)
161. Granner, D., Chase, L. R., Aurbach, G. D., Tomkins, G. M., *Science,* **162,** 1018–20 (1968)
162. Vaes, G., *Nature,* **219,** 939–40 (1968)
163. Pastan, I., Perlman, R. L., *J. Biol. Chem.,* **244,** 2226–32 (1969)
164. Jacquet, M., Kepes, A., *Biochem. Biophys. Res. Commun.,* **36,** 84–92 (1969)
165. Cheung, W. Y., *Anal. Biochem.,* **28,** 182–91 (1969)
166. Aurbach, G. D., Houston, B. A., *J. Biol. Chem.,* **243,** 5935–40 (1968)
167. Humes, J. L., Kuehl, F. A., Jr., *Federation Proc.,* **28,** 862 (1969)
168. Hepp, D., Challoner, D. R., Williams, R. H., *J. Biol. Chem.,* **243,** 4020–26 (1968)