STUDIES ON THE MECHANISM OF HORMONE ACTION

Nobel Lecture, December 11, 1971

by

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When I first entered the study of hormone action, some 25 years ago, there was a widespread feeling among biologists that hormone action could not be studied meaningfully in the absence of organized cell structure. However, as I reflected upon the history of biochemistry, it seemed to me there was a real possibility that hormones might act at the molecular level. For example, recalling how the biosynthesis of urea was elucidated, we find a period when urea synthesis could be studied only in the whole animal; later its formation was studied in perfused livers, then in liver slices, and was finally obtained in cell extracts. By analogy, it seemed that a systematic analysis of hormone action might also proceed from studies of the intact animal to isolated tissues and finally to soluble systems. In any event, that was the thought I had in mind when I began studying epinephrine and glucagon. At that time we referred to glucagon as the hyperglycemic-glycogenolytic factor (HGF) of the pancreas, not realizing that Burger and his colleagues had studied it many years earlier in Germany.

At this point I should acknowledge a large debt of gratitude to Professor Carl Cori. When I returned to St. Louis after medical service in World War II, I was undecided as to whether I should enter medical practice or go into research. Cori convinced me, not so much by anything he said so much as by his example, that research was the right direction for me to take. Although I have occasionally felt an urge to see patients more often, I have never really regretted this decision to stay in the laboratory.

Of course the intellectual environment in Cori's laboratory was highly conducive to this decision. I believe that kind of stimulating environment, with the necessary "critical mass" of young and talented investigators, with the opportunity for the free exchange of ideas, is an important ingredient in the making of scientific progress. Such an environment existed at Washington University then, just as it exists at Vanderbilt now, but it has always been rare. I regret that it threatens to become rarer still, at least in the United States, as a result of the continuing decline in the federal support of basic research.

Returning now to epinephrine and glucagon, studies on the glycogenolytic action of these hormones were attractive for several reasons. Their effects on glycogen breakdown and glucose production in the liver were rapid, large, and reproducible. Liver slices could be used and a number of slices could be prepared from the liver of one animal. In addition, the basic biochem-

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istry of glycogen breakdown had been established through the classical work of the Cori's and others, with phosphorylase, phosphoglucomutase, and glucose 6-phosphatase being the basic enzymes involved (1).

However, other enzymes were known to participate in glycogen metabolism, and at this stage even the possible hydrolysis of glycogen was thought to deserve consideration. Another basic point that had to be settled was whether or not the release of glucose might be a more primary event than glycogenolysis, since it seemed possible that glucose might be pumped out of the cell in response to the hormones, rather than overflowing as a result of increased glucose production. Measurement of glucose levels convinced us, however, that the increased glucose output resulted from an overflow phenomenon and not from an extrusion process in which glucose was actively transported from inside the cell (2).

Measurement of labelled intermediates, formed during incubation of rabbit liver slices in the presence of inorganic phosphate, led us to the conclusion that phosphorylase, rather than phosphoglucomutase or glucose 6-phosphatase, was rate-limiting in the conversion of glycogen to glucose, and that the hormones were acting to increase the activity of this enzyme (3). Glycogen loss was associated not only with an increase in glucose but with increased levels of both glucose l-phosphate and glucose 6-phosphate. An important problem complicating analysis at this stage was the accepted theory or assumption that phosphorylase catalyzed the *in vivo* synthesis of glycogen as well as its degradation. We knew that the hormones always stimulated glycogen breakdown rather than synthesis, so that even after phosphorylase activation had been demonstrated in response to the hormones (Fig. 1) an additional factor was suspected. One factor preventing phosphorylase from catalyzing glycogen synthesis *in vivo* is the high ratio of inorganic phosphate to hexose phosphate that normally prevails inside cells. Glycogen synthetase was not discovered until later (5) and we now know that its activity is decreased while phosphorylase activity is increased (6) as a result of the same basic reaction (7).

What was the nature of this reaction? Although phosphorylase activation by epinephrine or glucagon could be shown easily if intact cell preparations were studied, as in Fig. 1, virtually all response to the hormones was lost if the cells were broken. It was not clear, therefore, that a better understanding of phosphorylase activation would lead us very directly to a better understanding of how the hormones were acting. We nevertheless decided that this was our best approach. Wosilait and I were able to purify active phosphorylase from dog liver extracts, and then found another enzyme which would catalyze the inactivation of phosphorylase. Since the molecular weight did not seem to change during inactivation, we suspected that a relatively minor change might be responsible for the loss of activity, the loss of a phosphate group being only one of many possibilities. Had one assay been as easy to carry out as any other, it is probable that we would have done many more experiments than we did before discovering that the inactivation of phosphorylase was indeed accompanied by the loss of inorganic phosphate (Fig. 2). Thus our inactivating enzyme was shown to be a phosphatase.

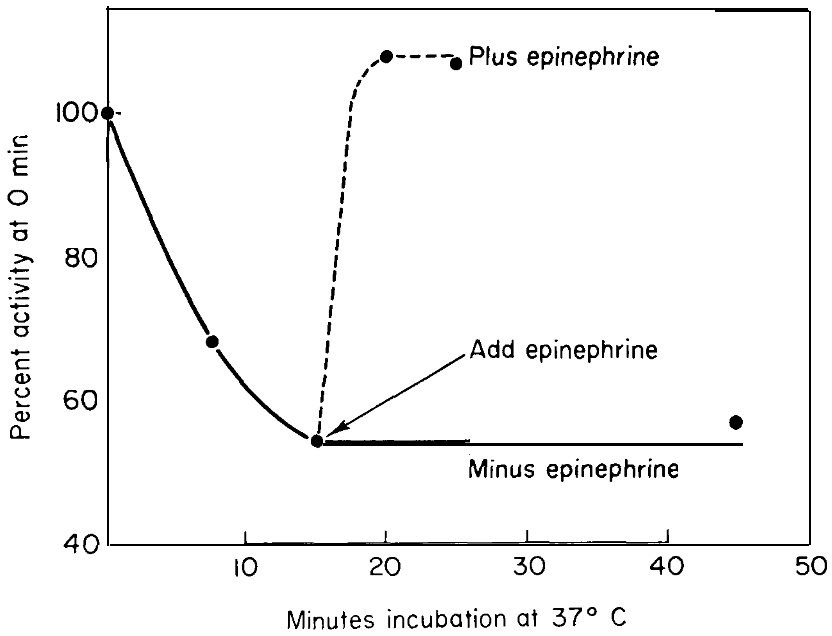

Fig. 1.
Effect of epinephrine on phosphorylase activity in dog liver slices. After incubating in a glycylglycine-phosphate buffer (pH 7.4) for the indicated times, the slices were homogenized and phosphorylase activity determined. From (4).

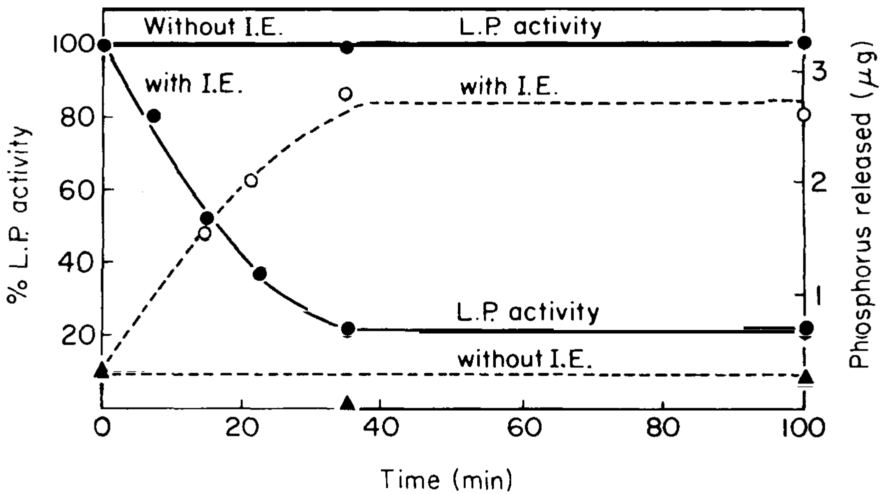

Fig. 2.
Effect of incubating with "inactivating enzyme" (I.E.) on liver phosphorylase (L.P.) activity and on the release of inorganic phosphate. Phosphate was measured in the supernatant fluid after TCA precipitation. From (8).

At about this time Ted Rall joined me, marking the beginning of a long and fruitful period of collaboration between us. The inactivation experiments had suggested that phosphorylase activation might be associated with phosphorylation of the enzyme molecule, and we were able to demonstrate this easily when liver slices were incubated in the presence of a phosphate buffer containing ^{32}P. Phosphate was rapidly incorporated into phosphorylase, and this was increased by the hormones, roughly in proportion to the degree of activation (4). It thus became clear that the concentration of active phosphorylase in liver represented a balance between inactivation by a phosphatase and reactivation by a process in which phosphate was donated to the protein. Krebs and Fischer (9) had meanwhile been studying phosphorylase activation in rabbit muscle extracts, and had demonstrated the requirement for ATP and Mg^{++}.

With this information in hand, we began to add hormones to our liver extracts in the presence of ATP and Mg^{++}. Progress was delayed for a time because we usually added fluoride to our homogenates, along with purified inactive phosphorylase. This seemed logical because we knew that fluoride would inhibit the phosphatase, and we wanted to inhibit this enzyme so as to preserve as much of the active phosphorylase that might be formed as possible. We now know that fluoride has another important effect in broken cell preparations - the stimulation of adenyl cyclase - and that this tends to mask the effect of hormones. Fortunately, we often incubated homogenates in the absence of fluoride, and in these experiments we began to see pronounced effects of epinephrine and glucagon (10). Our earlier discoveries - phosphorylase activation in slices, and the nature of the associated chemical change - were important landmarks, but not as exciting as finally establishing hormone effects in broken cell systems. Of course, we and others had often noted effects of hormone in this or that cell-free system, but, when pursued in greater depth, these effects had invariably proved to be non-specific. By contrast, the effects on phosphorylase activation appeared to be physiologically significant.

We then found that if we centrifuged our homogenates, to remove what we then thought of as cellular debris, the hormonal response was lost. It could be restored, however, by recombining the particulate fraction with the original supernatant (Fig. 3). We also found that if we incubated the particulate fraction with the hormones, a heat-stable factor was produced which could in turn activate phosphorylase when added to the supernatant fraction (10). The hormonal response was thus separated into two separate phases.

The next step was to identify the heat-stable factor. We showed that it was an adenine ribonucleotide (11) that could be produced from ATP by particulate preparations not only from liver, but also from heart, skeletal muscle, and brain (12). Lipkin and his colleagues had isolated the same compound from a barium hydroxide digest of ATP, and they later established its structure as adenosine 3', 5'-monophosphate (13) now commonly referred to as cyclic AMP or cAMP (Fig. 4).

Although cyclic AMP was not affected by a number of known phosphatases or diesterases, we did find an enzyme in animal tissues that rapidly

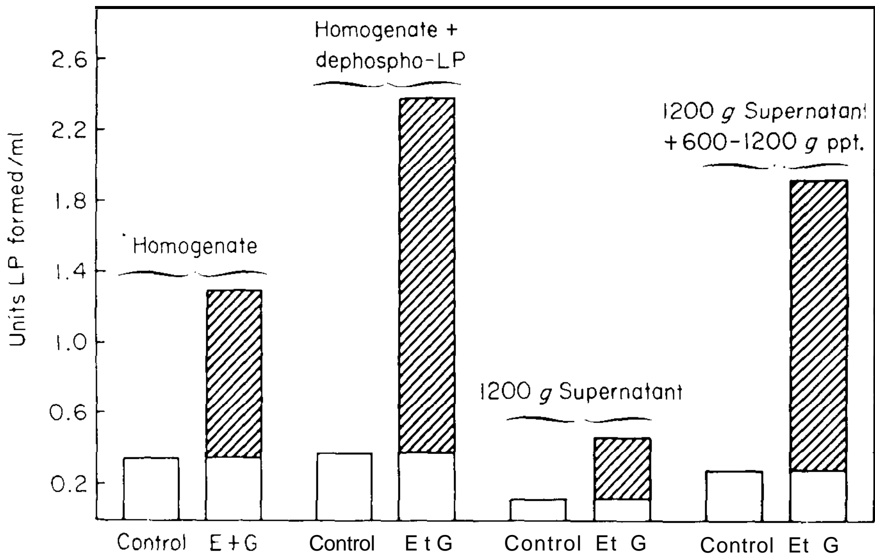

Fig. 3.
Effect of epinephrine and glucagon (E+) on phoshporylase activation in whole and fractionated cat liver homogenates. Inactive liver phosphorylase (dephospho-LP) was added where indicated, and the mixtures incubated with ATP and Mg^{++} in the presence and absence of the hormones. Phosphorylase activity was measured before and after 10 min incubation at 30° C. Each bar represents the amount of LP formed during this period. The effect of the hormones is indicated by the crosshatched areas. From (10).

inactivated it, with adenosine 5'-phosphate (5'-AMP) as the product of the reaction. It began to appear, therefore, that the concentration of cyclic AMP in tissues probably reflected a balance between two opposing reactions, formation from ATP, on the one side, and conversion to 5'-AMP, on the other. Our next series of studies were concerned with the enzymes responsible for these reactions.

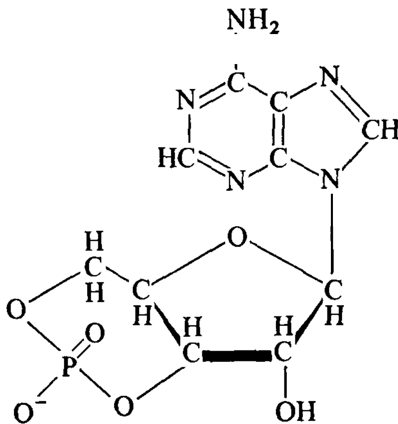

Fig. 4.
Structural formula of adenosine 3', 5'-monophosphate (cyclic AMP).

The activity in particulate fractions responsible for converting ATP to cyclic AMP was originally named adenyl cyclase, although a chemically more correct designation might have been adenylyl cyclase or perhaps adenylate cyclase. We established that this enzyme was widely distributed, not only in mammalian tissues but in a variety of other animal phyla as well (14), and pyrophosphate was shown to be another product of the reaction (15). In the meantime, Bill Butcher studied the phosphodiesterase responsible for catalyzing the breakdown of cyclic AMP, and he used this enzyme to establish the widespread occurrence of cyclic AMP in cells and fluids (16).

It had become clear by this point that epinephrine and glucagon increased the accumulation of cyclic AMP in liver homogenates by stimulating adenylyl cyclase, rather than by inhibiting the phosphodiesterase. Glucagon was shown to be more potent than epinephrine in this system (17), as was later shown to be the case in the intact liver as well (18). Epinephrine and other catecholamines were also shown to be effective in preparation of cardiac muscle (19). Then evidence began to accumulate to suggest that some of the effects of other hormones might be mediated by this same mechanism. For example, Haynes (20) showed that ACTH (but not glucagon or epinephrine) was capable of stimulating adenylyl cyclase in adrenal preparations, whereas ACTH was not active in the liver. In collaboration with Mansour and Bueding, we found that serotonin but not catecholamines was active in preparations of the liver fluke, *Fasciola hepatica* (21). Then TSH was found to stimulate cyclase in thyroid preparations (22). Developments to this stage were summarized in more detail in several review articles (2.3).

Although the cell membrane seemed the most likely source of adenylyl cyclase, our finding that the enzyme could not be detected in dog erythrocytes, which lacked nuclei, whereas it did occur in the nucleated erythrocytes of birds, forced us to consider the possibility that the nucleus might be the major source of the enzyme. Davoren thereupon designed and built a pressure homogenizer which caused extensive fragmentation of cell membranes with little damage to the nuclei. When this procedure was followed by density gradient centrifugation, it was possible to show that most of the adenylyl cyclase was not associated with nuclear material, but rather fractionated with what appeared to be fragments of the cell membrane (24).

As a result of these and other developments, we gradually began to think of cyclic AMP as a second messenger in hormone action, with the hormones themselves acting as first messengers (25). This concept is illustrated in Fig. 5. It would appear that different cells contain receptors for different hormones, and that an important result of the hormone-receptor interaction in some cells is to stimulate adenylyl cyclase, leading to increased levels of cyclic AMP. The cyclic AMP then acts intracellularly to alter the rate of one or more cellular processes. Since different cells contain different enzymes, the end result of the change in cyclic AMP will differ from one type of cell to the next, e. g., phosphorylase activation in hepatic cells, steroidogenesis in the adrenal cortex, and so on. This concept seemed to clarify, in our own minds at least, many of the phenomena which we and others had observed.

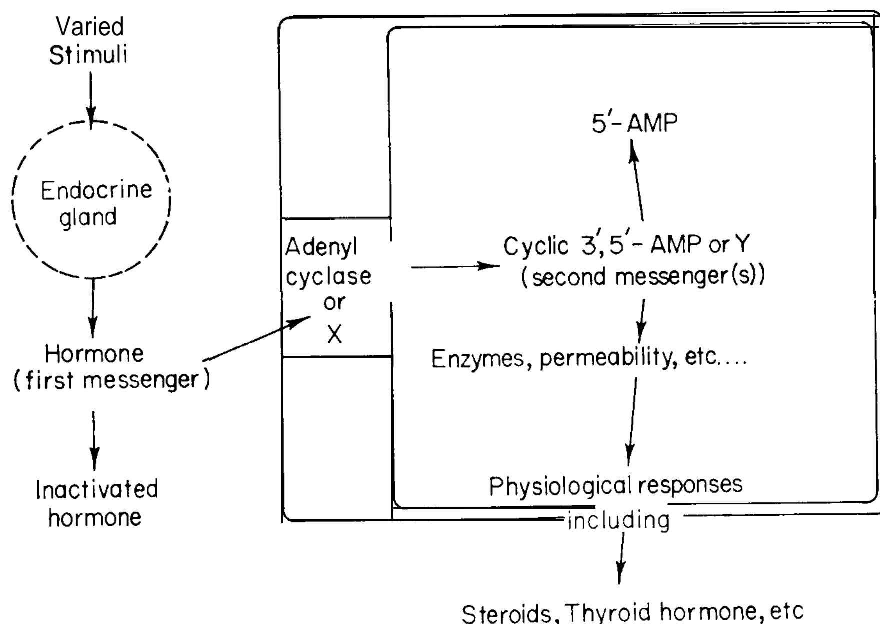

Fig. 5.
Schematic representation of the second messengers concept. From (26).

To further evaluate this hypothesis, and to help us determine which hormones might exert which of their effects by this mechanism, we decided to embark on a series of studies of different biological systems, with the following criteria in mind. First, if a given response to a given hormone is mediated by this mechanism, then adenylyl cyclase in the appropriate target cells should be stimulated by the hormone; conversely, hormones not producing the response should not stimulate the enzyme. Second, the level of cyclic AMP in intact tissues should change appropriately in response to hormonal stimulation; here we were thinking primarily of dose-response and temporal relationships. Third, we felt that drugs which inhibit phosphodiesterase, such as theophylline (16), should act synergistically with hormones that acted by stimulating adenylyl cyclase. Finally, it should be possible, at least in theory, to mimic the hormone by the application of exogenous cyclic AMP or one of its acyl derivatives. Of course we knew that most organic phosphate compounds such as cyclic AMP penetrated cells poorly if all, and the derivatives were synthesized by Theo Posternak in hopes that their more lipophilic nature might enable them to penetrate cell membranes more readily. Many of these derivatives were in fact more active than cyclic AMP, when applied to intact cells and tissues (27), but whether this can be related to better penetration remains to be established. These criteria have all had to be qualified, in the light of subsequent experience, but they did serve as useful guides for research. I will just mention briefly a few of the results obtained.

The positive inotropic response to epinephrine was of interest for many reasons, and was especially attractive to us because the heart contains only a limited number of different types of cells, with myocardial cells pre-

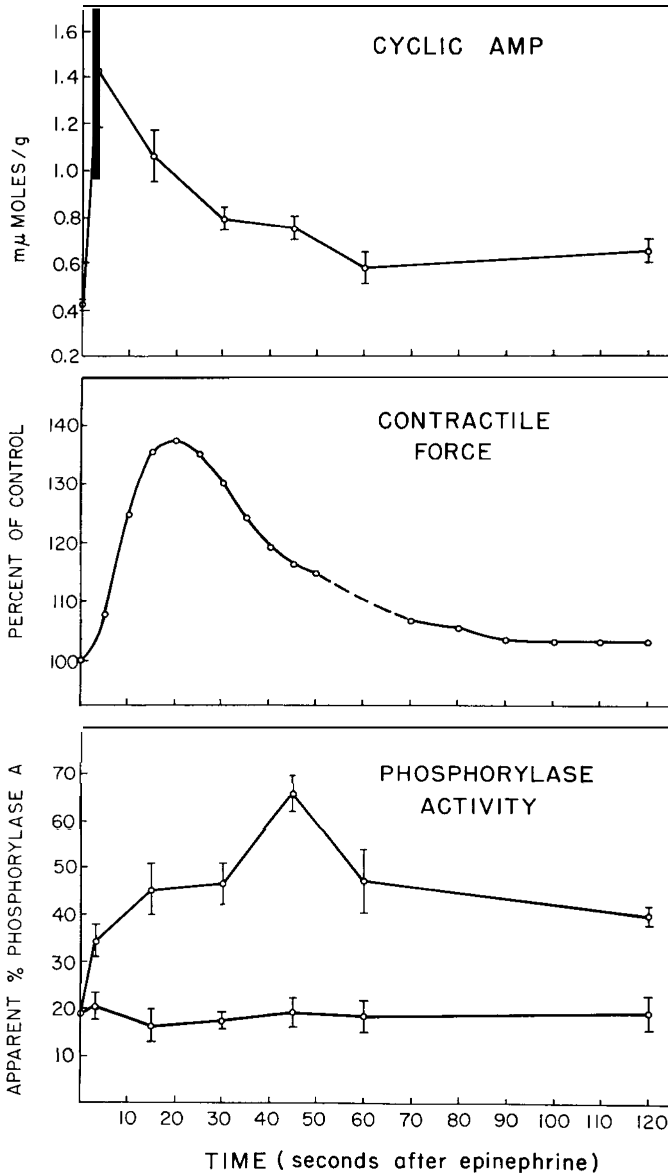

Fig. 6.
Effect of a single dose of epinephrine on cyclic AMP - contractile force, and phosphorylase activity in the isolated perfused working rat heart. Hearts were frozen at different times after injection of epinephrine. Lower curve in bottom panel shows lack of response to injected saline. From (28).

dominating. Hence we felt that biochemical studies of the heart would not be completely uninterpretable. The results of these studies have been summarized in more detail elsewhere (28-30) but an especially important result is shown in Fig. 6. We had previously found that the order of potency of several catecholamines in stimulating dog heart adenylyl cyclase was the same as others had reported for the inotropic response (19). Now we wondered if

cyclic AMP in the intact heart could possibly increase with sufficient speed to account for this response. As shown in Fig. 6, we found that cyclic AMP increased with extraordinary rapidity, reaching a 4-fold elevation within 3 seconds of a single injection of epinephrine, clearly fast enough to account for the increased force of contraction. We also found that the ability of several adrenergic blocking agents to prevent cyclic AMP accumulation in response to catecholamines correlated with their relative potencies as antagonists of the inotropic response. Our experiments with the rat heart were important because they represented our first attempt to measure tissue cyclic AMP levels while simultaneously monitoring a mechanical or functional response which could not be measured chemically. They also led us to a more serious consideration of the possibility that beta adrenergic effects in general might be mediated by cyclic AMP (29). Subsequent studies by ourselves and others have tended to support the view that the positive inotropic response (30, 31) and other beta adrenergic effects (32) are mediated by cyclic AMP. A related development has been the evidence that some alpha adrenergic effects result from a fall in the level of cyclic AMP (32, 33). However, the mechanism by which alpha receptors mediate this effect, and how applicable it will be to alpha adrenergic effects in general, are questions that remain for future research.

Lipolysis by rat adipose tissue was of special interest for other reasons, one being the large number of hormones capable of stimulating this response. Epinephrine, ACTH, and glucagon, for example, were known to be capable of stimulating adenylyl cyclase in other cells, and it seemed reasonable to suppose that they might also do this in adipocytes. Confirming this expectation, Fig. 7 shows that all three hormones caused a pronounced rise in cyclic AMP when added to isolated fat cells; this figure also shows the selective blockade of epinephrine by the beta adrenergic blocking agent pronethalol. Combinations of supramaximal doses of two or more hormones were never additive, leading to the conclusion that the hormones were all affecting the same cells, and presumably the same adenylyl cyclase. The result with pronethalol, however, showed clearly that they were interacting with separate receptors. Other results, summarized elsewhere (30, 34-36), disclosed that phosphodiesterase inhibitors acted synergistically with the hormones to increase cyclic AMP and stimulate lipolysis, and that exogenous cyclic AMP (or at least the acyl derivatives) would also stimulate lipolysis. We also found that insulin and certain prostaglandins were capable of suppressing the accumulation of cyclic AMP in fat cells, and presumably these substances owe at least part of their antilipolytic activity to this effect. More recently, we established that the relative potencies of a series of xanthine derivatives to enhance lipolysis correlated well with their ability to inhibit phosphodiesterase in fat cells (37). Our most recent finding in this area has been that fat cells produce an antagonist in response to hormones that stimulate cyclic AMP formation, and that this agent prevents further response to the hormones (38). Perhaps this antagonist plays an important role in the negative feedback regulation of many cells.

In collaboration with others, we also helped investigate the effects of vasopressin on the toad bladder (39) and the steroidogenic effects of LH and

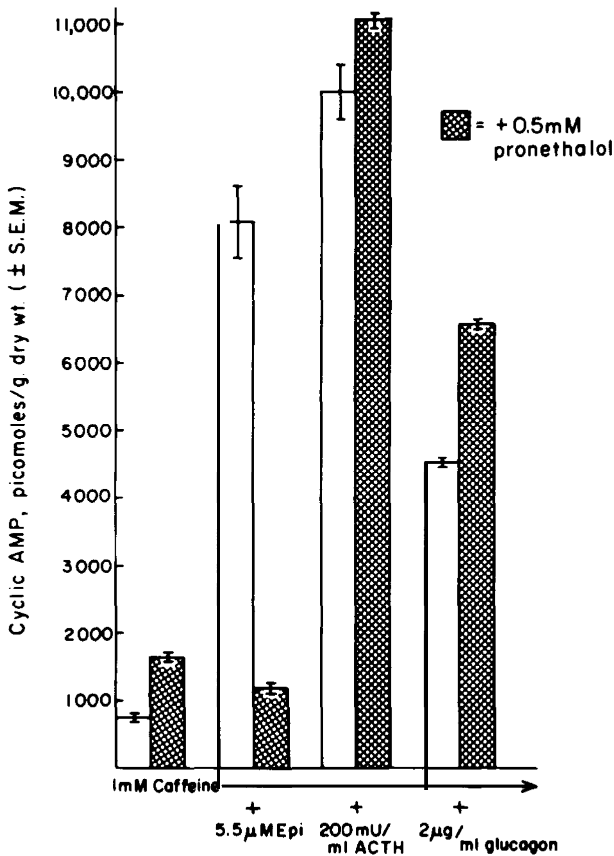

Fig. 7.
Effects of epinephrine (epi), ACTH, and glucagon, in the presence of caffeine, on cyclic AMP levels in isolated rat fat cells. Cells were incubated for 10 min in the presence of the hormones at the indicated concentrations, with and without pronethanol. From (34).

ACTH (40). It will not be possible here to discuss these and other results in detail. During recent years numerous investigators outside my own group have been active in this field, and progress has been correspondingly rapid. We attempted to summarize some of this progress in a recent monograph (41), and I might attempt a very brief recapitulation here.

Among hormones, we now know that vasopressin, ACTH, MSH, LH, parathyroid hormone, and TSH, besides epinephrine and glucagon, produce at least some of their effects by way of cyclic AMP. The production of certain other hormones, such as the steroid hormones, appears to be regulated by cyclic AMP. Thus the involvement of this nucleotide in endocrinology seems far-reaching. With reference to Fig. 5, we still do not understand how the hormone-receptor interaction leads to the stimulation of adenylyl cyclase. Robison developed an interesting model, according to which the protein component of the membrane adenylyl cyclase system was envisioned as being composed of two types of subunits (29): a regulatory subunit of which the recep-

tors were a part, facing the extracellular space, and a catalytic subunit with its active center in contact with the cytoplasm. However, this has been a difficult model to test experimentally. Hirata and Hayaishi (42) obtained a soluble cyclase from bacteria, but the system in eukaryotic cells may be more complex. Phospholipids appear to be important (43), and recently Rodbell and his colleagues (44) have implicated guanyl nucleotides in the regulation of adenylyl cyclase in liver. Fluoride, which we had used initially to inhibit phosphorylase phosphatase, stimulates cyclase activity in broken cell preparations of most tissues (14, 45), but we still do not understand the mechanism of this effect. There is now evidence to suggest that cyclase is held in the membrane in a restrained or inhibited state (46), and perhaps the hormones and fluoride act by relieving this inhibition. Some interesting developments have also been reported concerning the phosphodiesterase, which may turn out to be a more interesting enzyme (or perhaps I should say enzymes) than it originally appeared to be (47).

Cyclic AMP has been found to produce a large number of effects since our early studies on phosphorylase activation, and I have listed some of them in Table 1. We have not ourselves studied the mechanism of action of cyclic AMP within recent years, but substantial progress has been made by others. Walsh, Perkins, and Krebs (48) discovered a protein kinase which was stimulated by cyclic AMP, and which is responsible not only for activating phosphorylase but also for inactivating glycogen synthetase (7). This enzyme is composed of two subunits, a regulatory subunit to which cyclic AMP binds, and a catalytic subunit which is inhibited by the regulatory subunit (49). When cyclic AMP binds to the regulatory subunit, a dissociation occurs such that the catalytic subunit becomes freely active. Protein kinases are widely distributed in nature (50), and their activation (or deinhibition) by cyclic AMP may account for many of the known effects of this nucleotide.

Of course the various relations in the endocrine system cannot all be understood in terms of the simple concept illustrated in Fig. 5. I have already mentioned that some hormones, such as insulin, produce some of their effects by lowering the level of cyclic AMP, although we still do not understand the mechanism of this effect. The primary action of the steroid hormones appears to follow an entirely different pattern (51), although some interesting relationships between these hormones and those that act through cyclic AMP will probably be discovered. An interesting recent development has been the evidence that prolactin affects the mammary gland by increasing the amount of protein kinase; this protein, rather than cyclic AMP, appears to limit the rate of casein production by this tissue (52).

Cyclic AMP has also been found to play an important role in non-endocrine regulatory mechanisms. Makman (53) found that glucose could suppress the formation of cyclic AMP by *E. coli,* and we now know, from the work of Pastan and Perlman and others (54), that cyclic AMP plays an important role in regulating bacterial metabolism. Bonner and Konijn and their colleagues (55) found that cyclic AMP was needed for the aggregation and differentiation of certain species of cellular slime molds; in these organisms, interestingly

Table 1. Some Known Effects of Cyclic AMP[1]

Enzyme or Process Affected	Tissue Organism	Change in Activity or Rate
Protein kinase[2]	Several	Increased
Phosphorylase	Several	”
Glycogen synthetase	Several	Decreased
Phosphofructokinase	Liver fluke	Increased
Lipolysis	Adipose	”
Clearing factor lipase	Adipose	Decreased
Amino acid uptake	Adipose	”
Amino acid uptake	Liver and uterus	Increased
Synthesis of several enzymes	Liver	”
Net protein synthesis	Liver	Decreased
Gluconeogenesis	Liver	Increased
Ketogenesis	Liver	”
Steroidogenesis	Several	”
Water permeability	Epithelial	”
Ion permeability	”	”
Calcium resorption	Bone	”
Renin production	Kidney	”
Discharge frequency	Cerebellar Purkinje	Decreased
Membrane potential	Smooth muscle	Increased
Tension	” ”	Decreased
Contractility	Cardiac muscle	Increased
HCl secretion	Gastric mucosa	”
Fluid secretion	Insect salivary glands	”
Amylase release	Patorid gland	”
Insulin release	Pancrèas	”
Throid hormone release	Thyroid	”
Calcitonin release	Thyroid	”
Research of other hormones	Anterior pituitary	”
Histamine release	Mast cells	Decreased
Melanin granule dispersion	Melanocytes	Increased
Aggregation	Platelets	Decreased
Aggregation	Cellular slime molds	Increased
mRNA synthesis	Bacteria	”
Synthesis of several enzymes	”	”
Proliferation	Thymocytes	”
Cell growth	Tumor cells	Decreased

[1] References to most of these effects can be found in reference 41.
[2] Stimulation of protein kinase is known to mediate the effects of cyclic AMP on several systems, such as the glycogen synthetase and phosphorylase systems, and may be involved in many or even most of the other effects of cyclic AMP.

enough, cyclic AMP seems to function more as a first messenger than a second messenger. It is conceivable that cyclic AMP may at times act extracellularly in mammals too, since the proliferation of thymic lymphocytes was stimulated by exogenous cyclic AMP in concentrations similar to those that normally exist in plasma (56). Other evidence implicating cyclic AMP in the control of the immune response has been presented (57). Still another non-endocrine func-

tion of cyclic AMP may involve the regulation of vision (58) and possibly other senses. Our present understanding of the biological role of cyclic AMP is probably very small compared to what it will be in the future.

Most of our own research during recent years has centered around cyclic guanylic acid (cyclic GMP). Dr. Paul G. Hardman has been most active in this area. First found in urine by Price and his colleagues (59), it is still the only other 3', 5'-mononucleotide known to occur in nature. Our approach here has been the opposite, in a sense, of what it was when I began my studies of epinephrine and glucagon. Then we had a function, and found a nucleotide; now we have a nucleotide, and are trying to discover its function. We still seem a long way from success. Cyclic GMP has been found in all mammalian tissues studied and in many lower phyla (60); in most cells its level is about an order of magnitude lower than that of cyclic AMP, although some insects contain more cyclic GMP than cyclic AMP. Guanyl cyclase has been studied (61) and found to differ in several respects from mammalian adenylyl cyclase: it is partially soluble in most tissues (in contrast to the totally particulate adenylyl cyclase) it requires Mn^{++} for activity and it is not stimulated by fluoride. More importantly, from the standpoint of hormone action, we have so far been unable to detect an effect of any hormone on the activity of this enzyme.

Although cyclic AMP and cyclic GMP seem clearly to be produced by separate enzymes, it is less certain that they are metabolized separately. Both nucleotides can serve as substrates for the same enzyme, under some conditions (47), although there is evidence that in vivo they may be hydrolyzed largely by separate enzymes (62). An intriguing observation has been that physiological concentrations of cyclic GMP are capable of stimulating (by several fold) the hydrolysis of cyclic AMP by liver phosphodiesterase (63).

We have also studied the excretion of cyclic nucleotides, and here we have made some interesting observations (64, 65). Normal plasma levels of these nucleotides are low, in the order of 10^{-8}M, but urine ordinarily contains levels which are several orders of magnitude greater. Normal humans excrete about 2 to 9 μmoles of cyclic AMP per day, with the rate of excretion of cyclic GMP being about 30% of this. About 60% of the urinary cyclic AMP is derived from plasma by glomerular filtration with the balance coming from the kidney itself. Essentially 100% of urinary cyclic GMP is produced by glomerular filtration. We are still uncertain of which cells contribute how much to these extracellular values, but parathyroid hormone (PTH) seems to have an important influence in the case of cyclic AMP. Very striking increases in urinary cyclic AMP are seen after the injection of PTH in man, while the infusion of calcium chloride, which suppresses PTH secretion, leads to the opposite effect. This is shown for four patients in Fig. 8, which also illustrates the unexpected finding that urinary cyclic GMP levels were *increased* in response to calcium infusions. Other differential effects on these nucleotides have been noted. In rats, for example, hypophysectomy lowered the excretion of cyclic GMP to less than half of normal while reducing only slightly the excretion of cyclic AMP (64); conversely, the injection of glucagon greatly in-

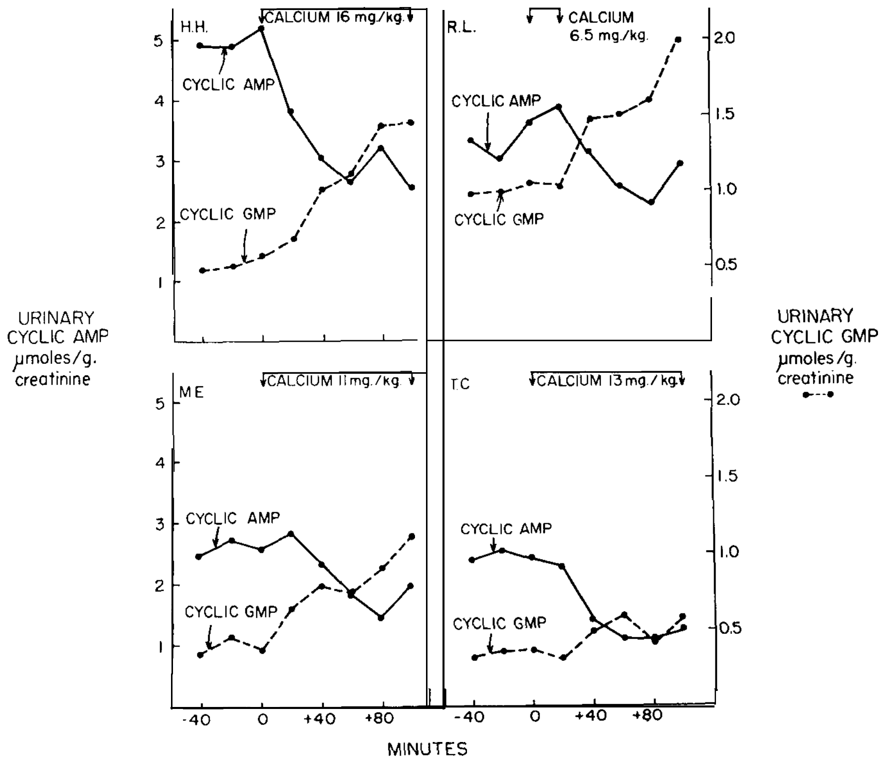

Fig. 8.
Comparison of urinary cyclic AMP and cyclic GMP in response to calcium infusion. Subjects received from 0.33 to 0.50 mg/kg/min of Ca^{++} during the first 15 min of the infusion. From (66).

creased cyclic AMP excretion with no effect on cyclic GMP. Another interesting finding was made by Goldberg and his colleagues (67); acetylcholine increased the level of cyclic GMP while reducing cyclic AMP in the isolated perfused rat heart.

These and other findings have led to the conclusion that the biological role of cyclic GMP, if it has such a role, must be quite different from that of cyclic AMP. An observation which seemed initially at variance with this conclusion was that exogenous cyclic GMP was almost as potent as cyclic AMP in stimulating glucose production by the isolated perfused rat liver. This was surprising because we had previously found (15) that cyclic GMP was less effective than cyclic AMP by several orders of magnitude in stimulating phosphorylase activation in dog liver extracts. Our first thought, that cyclic GMP might be inhibiting phosphodiesterase in the rat liver, thereby allowing cyclic AMP to accumulate, was found to be incorrect. We then found that exogenous cyclic GMP accumulated in the liver to a much greater extent than cyclic AMP, and this appears to account for its unexpected potency (68). We are continuing our studies to define a role for cyclic GMP. Many possibilities have yet to be explored, and whether cyclic GMP will prove to be as interesting as cyclic AMP remains to be seen.

I might turn briefly to the role of cyclic AMP in human disease. Because cyclic AMP serves primarily a regulatory function, it seems likely that a great variety of disorders may be related to defects in the formation or action of this nucleotide. One of the first examples to be studied was pseudohypoparathyroidism, a rare hereditary disease in which PTH appears incapable of stimulating cyclic AMP formation in target tissues in vivo (69); other hereditary disorders in which cyclic AMP has been implicated would include bronchial asthma (70), diabetes mellitus (71), and certain affective disorders (72). Some bacterial infections may involve cyclic AMP, and an especially interesting example has been cholera. The toxin responsible for this disease produces an apparently irreversible increase in adenyl cyclase activity, leading to prolonged high levels of cyclic AMP in intestinal epithelia; this in turn leads to the debilitating loss of fluids and electrolytes characteristic of this disease (73). As a final and possibly very important example, there is now evidence that defective cyclic AMP formation may be involved in the growth of tumors (74). To date, our knowledge that cyclic AMP may be involved in certain diseases has not lead to improved methods of therapy. It seems likely, however, that such methods will evolve as more detailed information accumulates. Several drugs already in use appear to act through cyclic AMP mechanisms.

In this lecture I have attempted to summarize some of my own research on the mechanism of hormone action, together with some results by others which have led to our present understanding of the role of cyclic AMP.

In conclusion I wish to suggest, or plead, that all of us exert a small amount of effort to stimulate interest in biological and medical research. A life in research can be a most enjoyable life with many frontiers to explore. In addition we need research to understand man and his ailments. I believe we are reaching a stage where research will be more and more helpful to man.

These points seem very obvious to me, and until the last few years they have appeared so obvious and simple that they were not worthy of mention. In these times, however, we may need to state and restate such simple points.

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77. Although the food supply is probably not the most important factor limiting the populations of most species. See, for example, Christian, J. J., Biology of Reproduction 4, 248, (1971).
78. I am grateful to the many colleagues who helped in this research. James W. Davis has worked with me for over 15 years. Other important contributions were made by Drs. W. D. Wosilait, T. W. Rall, R. W. Butcher, G. A. Robison and J. G. Hardman, among others. I am further grateful to the National Institutes of Health and to the American Heart Association for their support over the years. I am indebted to Rollo Park not only for his friendship but for this creation of an ideal research environment.