of *p*-bromoaniline was spotted and run through the solvent system several times. There was no acetylation.

When the organisms were incubated with *p*-bromoaniline at concentrations of 1, 10, and 20 μg/ml, all of the organisms tested converted the aniline to the acetanilide. No starting aniline or the azobenzene was observed after incubation for 7 days. Thus, the acetylation of substituted anilines in soils may be competitive with the oxidative coupling to azobenzenes, at least in the case of bromine-containing aniline from the phenyl ureas. Unless the coupling reaction is very fast or the aniline concentration is very high, little or no azobenzene may be formed. The removal of the anilines from the soil by acetylation should be advantageous in the metabolic degradation of urea herbicides because the acetanilides are usually less toxic than either the corresponding aniline or azobenzene.

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19 January 1970; revised 10 March 1970

Adenosine 3′,5′-Monophosphate, Adrenocorticotropic Hormone, and Adrenocortical Cytosol Protein Synthesis

Abstract. *In cultures of mouse adrenocortical tumor cells (Sato's minimal deviation Y-1 clonal strain), the acceleration of steroid biosynthesis after exposure to adrenocorticotropic hormone or cyclic adenosine 3′,5′-monophosphate is maximum within 15 to 60 minutes and precedes any significant increase in labeling of protein with [4,5-^{3}H]leucine. However, when cytosol proteins are separated by acrylamide-gel electrophoresis, rapid changes in the amount and labeling of several protein fractions are evident in less than 30 minutes and are no longer evident within 60 minutes. This finding supports the proposal that the effects of tropic hormones and their intracellular mediators involve rapid selective effects on protein synthesis.*

Adenosine 3′,5′-monophosphate (cyclic AMP) is the probable intracellular mediator of many of the actions of hormonal polypeptides and amines on their target cells. The role of the nucleotide in the steroidogenic effect of adrenocorticotropic hormone (ACTH) on adrenal cortex seems well established (*1*). The activity of membrane-associated adenyl cyclase is stimulated by ACTH, increasing the conversion of adenosine triphosphate to cyclic AMP. An increased concentration of cyclic AMP stimulates the enzymatic conversion of cholesterol to Δ^5-pregnenolone, the rate-limiting enzymatic step in adrenal steroidogenesis (*2*). The nature of the step or steps between the increase in cyclic AMP and the cleavage of the cholesterol side chain is still obscure.

That protein synthesis may be involved either directly or indirectly is suggested by the fact that (i) pharmacologic inhibitors of protein synthesis can interfere with the rapid effect of ACTH or cyclic AMP on steroid biosynthesis (*3*) without preventing the action of ACTH on adenyl cyclase (*4*), and (ii) there is an apparent lack of a direct effect of cyclic AMP on a side-chain cleaving system in mitochondria (*5*). This evidence has been difficult to evaluate because there has been no proof that the action of the inhibitors was exclusively derived from decreased protein synthesis (*6*). In addition, there has been no previous evidence that ACTH affects adrenocortical protein synthesis during the period of its initial effects on steroidogenesis. Evidence that ACTH and cyclic AMP actions involve protein synthesis must first include such a demonstration; second, cyclic AMP must be shown to have an ACTH-like effect on side-chain cleavage in adrenocortical mitochondria.

We studied the effects of exogenous ACTH and cyclic AMP on a clonal strain of mouse adrenocortical tumor cells (*7*) in monolayer culture. These cells respond to ACTH with a rapid increase in adenyl cyclase activity (*8*) and to both ACTH and cyclic AMP with a rapid increase in steroidogenesis (*9*). We added 5 munit of ACTH or 1.0 μmole of cyclic AMP per milliliter of tissue culture medium, amounts which produced roughly the same steroidogenic effect in cultures incubated for 2 hours or less. The effect of ACTH on the rate of steroid production [number of micrograms of the major steroidal products (*9*) per 10^6 cells per hour] was maximum in less than 30 minutes (Fig. 1). The difference in time of the peak effect of cyclic AMP from that of ACTH was accompanied by extreme variability of the steroidogenic rate in experiments where the cultures were incubated with cyclic AMP, and can be attributed to the relative impermeability of most plasma cell membranes to the nucleotide (*1*).

To investigate alterations in protein synthesis during the acceleration of steroid synthesis after addition of ACTH or cyclic AMP, we also added 2.5 μc of [^{3}H]leucine per milliliter of tissue culture medium (*10*). Tumor cells were harvested in an ice-cold saline solution, rinsed three times to wash away medium proteins, subjected to osmotic shock, and homogenized; they

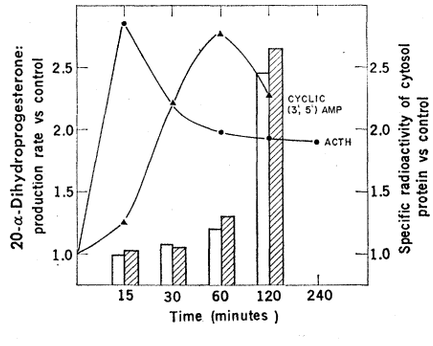

Fig. 1. Effects of ACTH and cyclic AMP on steroid production and on the specific [^{3}H]leucine radioactivity of cytosol protein in adrenocortical tumor cells. Closed circles indicate the ratios of 20-dihydroprogesterone production (micrograms of steroid per 10^6 cells per unit time) in cells exposed to 5 munit of ACTH per milliliter of culture for various periods (*9*). Closed triangles indicate those exposed to 1.0 μmole of cyclic AMP per milliliter. Bars indicate the ratios of the specific radioactivity of the cytosol protein in cells treated with ACTH to untreated cells (open bars), and of cells treated with cyclic AMP to controls (closed bars). The values shown represent averages from five different experiments.

were then separated by ultracentrifugation in 0.25*M* sucrose solution into nuclear and cytoplasmic subcellular fractions. We measured the specific radioactivity of the protein precipitated from each fraction by trichloroacetic acid (TCA). We also examined the distribution of cell fraction proteins by analytical acrylamide-gel electrophoresis (*11*). After being fixed in 12.5 percent TCA the proteins were stained with Coomassie brilliant blue. With small amounts of protein—for example, less than 15 μg per gel used here—protein concentration was linearly related to the intensity of staining (*12*).

Within 15 to 30 minutes after addition of ACTH, the synthesis of only two protein fractions of the 105,000*g* cytoplasmic supernatant "cytosol" fraction appeared to be altered (Fig. 2). The amount of fraction 11, indicated by absorbance of the stained protein band at 5500 Å, was increased and that of fraction 14 was decreased within 15 minutes of ACTH addition. Cells harvested 15 minutes after addition of ACTH were exposed to [^{3}H]leucine for 15 minutes before addition of the hormone, so that exposure to isotope was for 30 minutes, as in all the other groups. The first 15 minutes of labeling was thus under control conditions. The radioactivity in the region fractions 10 and 11 was nevertheless increased within 15 minutes after addition of ACTH and decreased in the region of fraction 14. That the labeling and quantitative changes were similar indicates selective effects on the synthesis of fractions 11 and 14, and is evidence against an effect on stabilization.

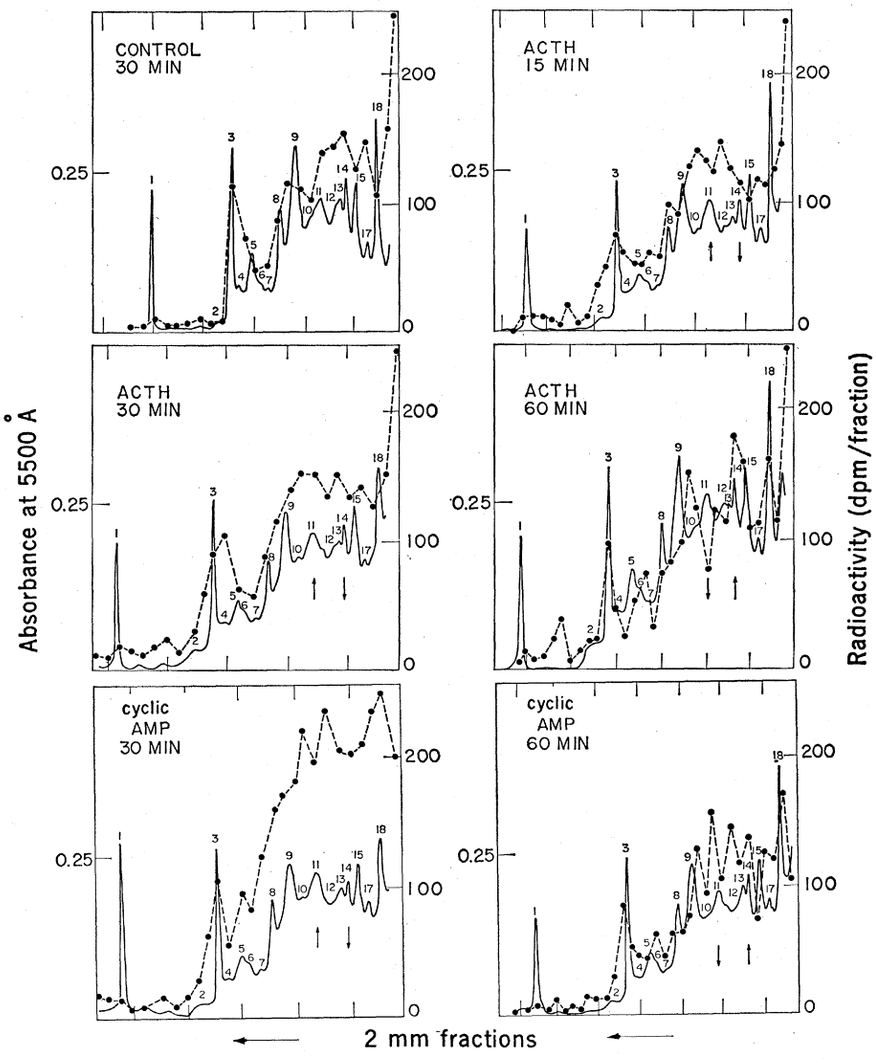

Fig. 2. Acrylamide-gel electrophoresis of adrenocortical tumor cell cytosol proteins: effects of ACTH and cyclic AMP. The cells in each group were exposed to [^{3}H]leucine for 30 minutes before being harvested. Approximately 7 μg of protein per gel cylinder was subjected to disk electrophoresis through 7.5 percent acrylamide at *p*H 8.9 for 75 minutes (2.0 ma per gel); the protein was fixed with 12.5 percent trichloroacetic acid and stained with Coomassie brilliant blue. The direction of migration is right to left in each electropherogram. Solid lines represent the absorbance at 5500 Å determined with a Gilford linear gel scanner and model 2000 spectrophotometer. Frozen gel cylinders were sliced into 2-mm sections and the radioactivity of each section, indicated by broken lines, was determined by liquid scintillation counting. See (*11*).

The areas under each peak, determined by planimetry, have been taken to represent the quantity of each fraction (*13*). In five different experiments, fraction 11 contained an average 10.2± 0.37 percent (± standard error) of the total protein in the acrylamide gel and fraction 14 contained 4.8 ± 0.21 percent. In Fig. 3, the effects of ACTH and cyclic AMP are given as percentages of these control values. Quantitative changes 15 minutes after addition of ACTH were highly significant for both fraction 11 ($P < .001$) and fraction 14 ($P < .005$).

Cells in which steroidogenesis was unaffected 15 minutes after exposure to cyclic AMP did not exhibit these changes. However, after 30 minutes, when apparently enough cyclic AMP had entered the cell to accelerate the rate of steroid biosynthesis, the radioactivity and amount of each fraction was affected in the same way as it was 15 minutes after addition of ACTH (fraction 11, $P < .001$; fraction 14, $P < .005$). The general increase in protein radioactivity seen in the electropherogram 30 minutes after the addition of cyclic AMP (Fig. 2) was only occasionally observed, and bore no relation to the selective changes in fractions 11 and 14. It is possible that aminoacyl-tRNA synthetase was transiently activated by the nucleotide, an effect recently seen in reticulocytes (*14*).

The rate of steroidogenesis 30 minutes after addition of ACTH was almost as high as at 15 minutes, and the changes in fractions 11 and 14 were still evident (Fig. 2). In some other experiments the effect on the quantity of fraction 11 had disappeared by 30 minutes, although fraction 14 was still diminished in amount and radioactivity. Sixty minutes after addition of either ACTH or cyclic AMP both changes had disappeared, although steroidogenesis still could continue at a high rate for several hours without further stimulation. The transiency of changes in the two protein fractions, and evidence of much longer survival

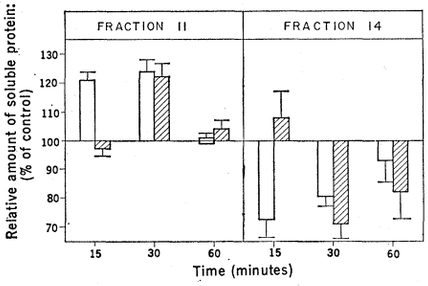

Fig. 3. Quantitative effects of ACTH and cylic AMP on fractions 11 and 14. Total area of the stained protein was fractionated by acrylamide-gel electrophoresis and the area under each peak was determined by planimetry (*13*). The change in the relative contribution of each fraction is shown for cells harvested 15, 30, and 60 minutes after exposure to ACTH (open bars) and cyclic AMP (closed bars). Standard errors are indicated by the line extending from each bar; there were at least five electropherograms in each group. The statistical significance of differences was estimated with Student's *t*-test for independent means (*18*).

of stimulatory amounts of either hormone or nucleotide in the tissue culture medium, both suggest that protein synthesis is involved in the induction of, rather than the maintenance of, increased steroidogenesis. Since there was no effect on fractions 11 and 14 when steroid production was increased by addition of reduced nicotinamide-adenine dinucleotide phosphate or Δ^5-pregnenolone to the culture medium, the possibility that changes in intracellular steroid concentrations were responsible for the effects on protein synthesis can be ruled out.

There is already evidence that cyclic AMP can selectively affect protein synthesis in its mediation of glucagon and catecholamine effects on liver (*15*) and in the synthesis of galactosidase in *Escherichia coli* (*16*). Our findings show that the action of cyclic AMP in steroidogenic cells also includes specific effects on protein synthesis. The discovery of rapid positive and negative changes of only a few protein fractions, before any effect on amount or labeling of total cytosol proteins or total cell proteins, is consistent with our observations of the effect of ACTH on guinea pig adrenal cortex in vivo (*17*) and with Farese's concept of two labile adrenocortical cytosol proteins, one a promoter of cholesterol side-chain cleavage and the other an inhibitor (*5*). The proteins of fractions 11 and 14 may have such effects on an adrenocortical mitochondrial system.

Discovery that the principal intracellular mediator of ACTH action and the hormone itself have similar effects on protein synthesis (when the lag in the steroidogenic effect of the nucleotide is accounted for) encourages the suspicion that these two protein fractions play a role in regulating the induction of increases in steroidogenesis. Perhaps target cells for other polypeptide hormones will also show such changes when similarly examined. Evidence that labile proteins are important in the control of steroid biosynthesis is still, however, no more than circumstantial.

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6 November 1969; revised 9 February 1970

Colcemid Sensitivity of Fission Yeast and the Isolation of Colcemid-Resistant Mutants

Abstract. *Cell division of the fission yeast,* Schizosaccharomyces pombe, *is reversibly inhibited by the antimitotic agent Colcemid* (N-*deacetyl*-N-*methylcolchicine) in nutrient medium. Cell growth continues until all cells become nonseparating cell doublets in a V configuration. Mutants have been isolated capable of uninhibited growth in the presence of concentrations of Colcemid mycostatic for the parent strain.*

Biochemical and physiological investigations on cell division and mitosis have benefited from the use of agents which interact with cellular components needed for normal chromosome segregation. The colchicine family of alkaloids has wide use in such studies as a mitotic poison for animal and plant cells (*1*). Use of radioactive colchicine has allowed identification and partial purification of a protein component of the microtubules of the mitotic apparatus which binds the drug (*2*). It seemed reasonable to try to apply these drugs to a study of the mitotic apparatus of a microbial eucaryotic cell. The species desired should combine features of chromosome segregation of higher cells with the genetic flexibility of microbes. Initially, several microbial eucaryotes appeared to offer equivalent advantages of ease of cultivation and genetic analysis.

Colchicine induces abnormal cell morphology in *Chlamydomonas eugametos* (*3*) and inhibits regeneration of flagella in *C. reinhardi* (*4*). It inhibits and then synchronizes cell division (*5*) and inhibits regeneration of cilia in *Tetrahymena pyriformis* (*6*). We had elected to work with the fission yeast, *Schizosaccharomyces pombe,* because its mode of cell division seemed to favor the detection of aberrancies of this process and because of its suitability for genetic analysis (*7*). We now report the sensitivity of this yeast to colchicine and its *N*-deacetyl-*N*-methyl derivative Colcemid (Ciba), and the isolation of drug-resistant mutants.