RECENT PROGRESS IN HORMONE RESEARCH

The Proceedings of the 1961 Laurentian Hormone Conference

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GREGORY PINCUS

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PREFACE

From time to time the Laurentian Hormone Conference leaves its Canadian meeting place and journeys westward. In September, 1961, this journey ended at Hoberg's in California where, at appropriate altitude and amid pine grove greenery, a meeting was held.

As indicated in the table of Contents, aspects of four major research areas were presented and discussed. The first of these, concerned with hormonal polypeptides and their functional activities, is initiated by Dr. Li and Dr. Hofmann and Dr. Yajima, who review especially their contributions to the burgeoning field of pituitary polypeptide synthesis. Perhaps the most notable finding is that certain properties of naturally-occurring polypeptide hormones may reside in much less complicated synthetic molecules. Dr. Rudman and his colleagues have marshalled their evidence for the existence of what may be a new pituitary hormone concerned with lipid mobilization. The skillful application to clinical material of methods for determining variations in corticotropin secretion is presented in precise detail by Dr. Liddle, Dr. Island, and Dr. Meador. Finally, the case for angiotensin as a renal hormone is knowledgeably argued by Dr. Page and Dr. Bumpus.

In the section concerned with thyroid hormones, Dr. Greer reviews expertly the effects, nature, and occurrence of goitrogens. These agents have yielded much information pertinent to the physiology of the thyroid gland and of thyroid hormone secretion. Dr. Tata's review is concerned essentially with the present-day status of investigations concerned with the mechanism of thyroid hormone action. It is clear from this paper and the discussion following it, that this is a very fruitful field in present-day endocrinology and undoubtedly will continue to yield important and informative data.

It is especially gratifying to be able to have a discussion of parathyroid hormone, which for many years has been a rather neglected endocrine agent. Probably one of the chief stimulants to the renaissance of this field is the work of Dr. Rasmussen and Dr. Craig on the isolation and chemical identification of the hormone. When, to this, is added their careful analysis of biochemical and physiological effects, and then the discussion of Dr. Gordan and his colleagues on the clinical endocrinology, an interesting and well-rounded story results.

In the final section of this book concerned with steroid hormones, one of the classic problems of steroid analysis, namely the measurement of urinary estrogens in man, is considered by Dr. Brown and Dr. Matthew, who present, also, some applications of the now widely used analytical methods of Dr. Brown. The paper by Dr. Jensen and Dr. Jacobson is a skillful presentation of data essential to the studies of the mechanisms of estrogen action. Particularly striking is the use of isotopes and the deductions that may be made from the manner in which they are localized. We are grateful to Dr. Emmens for the careful resumé of his most interesting work on estrogen inhibitors and antagonists. The contrast between the action of competitive

inhibitors and specific antagonists is both physiological and biochemically meaningful and may indeed offer additional clues to the mechanism of estrogen action. For the two final papers concerned with steroid hormones, we are indebted to Dr. Yielding and Dr. Tomkins for an account of their investigations of steroid-glutamic dehydrogenase interactions, and to Dr. Feigelson and his colleagues on the role of hormones in rat liver tryptophan pyrrolase induction. Each of these papers illustrates a special type of approach to the hormone-enzyme problem. Again, this is a field of very active present-day investigation and will undoubtedly continue to yield rich harvests.

ACKNOWLEDGMENTS

As in the past, the combination of scientific meeting and discussion with the chance of relaxation in the mountain climate proved to be most pleasing to the members of the Conference. They are especially indebted to the Hoberg family and to Mr. Ernest Summers for unfailing courtesy and helpfulness.

Contributions to the support of the Conference were made by the following companies: Abbott Laboratories; Armour Pharmaceutical Company: Ayerst Laboratories; Baxter Laboratories, Inc.; Ciba Company Limited; Ciba Pharmaceutical Products; Cutter Laboratories; Charles E. Frosst & Co.; General Mills, Inc.; Hoffmann-LaRoche, Inc.; Lederle Laboratories Division, American Cyanamid Co.; Lilly Research Laboratories; Mattox and Moore, Inc.; Mead Johnson & Co.; Merck & Co., Inc.; Wm. S. Merrell Co.; Nordic Biochemicals; Organics, Inc.; Organon, Inc.; Ortho Research Foundation; Parke, Davis & Co.; Chas. Pfizer & Co.; Pfizer International; Riker Laboratories; J. B. Roerig & Company; Schering, A. G.; Schering Corporation; Searle Chemicals, Inc.; G. D. Searle & Co.; Smith Kline & French Laboratories; Smith, Miller & Patch, Inc.; E. R. Squibb & Sons of Canada; The Squibb Institute for Medical Research; Strasenburgh Laboratories; Sterling-Winthrop Research Institute; Syntex, S.A.; The Upjohn Company; Warner-Chilcott Laboratories; Warner-Lambert Research Institute; Wyeth Institute for Medical Research. Because of the generous support made by these sponsors, the Conference was able to have as special guest speakers from abroad Dr. Jamshed Tata from Sweden, and Dr. Clifford W. Emmens from Australia.

The Committee on Arrangements is indebted to Drs. A. Wilhelmi, F. C. Bartter, Jan Wolff, P. L. Munson, G. E. W. Wolstenholme, R. L. Noble, and L. L. Engel for acting as chairmen of the various sessions. Mrs. Jeanne Arentsen and Mrs. Gertrude Leary of Dr. G. S. Gordan's staff generously volunteered their services as secretaries to the Conference, together with our regular Conference secretaries, Mrs. Jacqueline C. Foss and Mrs. Mina Rano. The subject index of this volume has been ably prepared by Mrs. L. P. Romanoff.

Shrewsbury, Massachusetts
March, 1962

GREGORY PINCUS

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Synthesis and Biological Properties of ACTH Peptides*

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I. INTRODUCTION

Investigations that have been conducted during the last decade on the isolation, physicochemical characterization, biology, immunochemistry, and synthesis of pituitary hormones, have given us some important general concepts in the field of molecular endocrinology: (a) Preparations of the same hormone isolated from glands of various species are not necessarily chemically identical, even in those instances where no differences in biological behavior have been observed. (b) Hormonal activities do not depend upon the integrity of the whole molecule. Activities can be dissociated either by chemical modification of the molecule or by chemical synthesis of different portions of the molecule. (c) Two hormones manifesting divergent physicochemical behavior may possess overlapping biological activities. (d) Two hormones that originate from different parts of the gland may have a common structure and, in turn, may exercise similar biological functions. (e) Not all hormones are species specific as far as their immunological behavior is concerned. Some are potent antigens and others are poor antigens. (f) A tropic hormone can exhibit activity in the absence of its target organ; that is, it can produce extra-endocrine responses. (g) Preparations of a particular hormone, no matter what the species, may be biologically active in certain animals, but some may not evoke any biological response at all in other animals. (h) The injection of a hormone derived from a single species into different experimental animals may elicit a different profile of response on the part of the same target organ.

I shall now devote my discussion to the structure and function of ACTH. You will see that a number of the general concepts outlined above are derived from studies of this very interesting molecule.

II. ISOLATION AND STRUCTURE OF ACTH†

A polypeptide possessing ACTH activity has been isolated in a pure state from sheep pituitaries by Li *et al.* (53) and from pig pituitaries by Bell *et al.*

* Paper XXVI of Adrenocorticotropin (ACTH) series.

† Earlier studies on the chemistry of ACTH have been extensively reviewed by Astwood *et al.* (1) and Hays and White (29) at previous conferences.

Structure of bovine ACTH:

Ser	Tyr	Ser	Met	Glu	His	Phe	Arg	Try	Gly	Lys	Pro	Val	Gly	Lys	Lys	Arg	Arg	Pro	Val
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

Lys	Val	Tyr	Pro	Asp	Gly	Glu	Ala	Glu	Asp	Ser	Ala	Glu(NH_2)	Ala	Phe	Pro	Leu	Glu	Phe
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39

Species	Laboratory*	Amino acid residue in position								
		25	26	27	28	29	30	31	32	33
Pig	American Cyanamid Company	Asp	Gly	Ala	Glu	Asp	Glu(NH_2)	Leu	Ala	Glu
Sheep	University of California	Ala	Gly	Glu	Asp	Asp	Glu	Ala	Ser	Glu(NH_2)
Beef	University of California	Asp	Gly	Glu	Ala	Glu	Asp	Ser	Ala	Glu(NH_2)

*See references (37,49,52)

FIG. 1. Structural differences among adrenocorticotropins isolated from pig, sheep, and beef pituitary glands.

(2), both in 1954; from beef pituitaries by Li and Dixon (48), in 1956; and from human pituitaries by Lee *et al.* (43), in 1959. The complete structures of the porcine and ovine hormones were reported by Howard (37) and by Li (52) and their co-workers, respectively, in 1955, and the complete structure of the bovine ACTH was described by Li *et al.* (49), in 1958. The ovine, porcine, and bovine adrenocorticotropins are single-chain polypeptides composed of 39 amino acids, and they have been calculated to have a molecular weight of approximately 4500. Figure 1 summarizes the amino acid sequences in these three adrenocorticotropins. It can be noted that the only difference in amino acid sequence between the porcine and ovine hormones appears to be one more leucine in the porcine, and one more serine in the ovine, hormone; this difference is manifested at positions 31 and 32 of the amino acid chain, where alanine and serine appear in the ovine hormone and leucine and alanine in the porcine. Although there are no differences in amino acid composition between the ovine and bovine hormone, a difference in arrangement of the amino acids in a small portion of the polypeptide chain appears between positions 25 and 32. Furthermore, the amino acid residue at position 33 in the porcine hormone is glutamic acid, whereas the same position in either the bovine or ovine ACTH molecule is occupied by glutamine. From these various structural considerations, it may be inferred that removal of that portion of the COOH-terminal sequence which includes positions 25–39 will not change the biological properties of ACTH. Indeed, it was shown that when the porcine (3) or ovine (10) hormones are subjected to hydrolysis with the enzyme pepsin, the COOH-terminal undecapeptide is released with no loss of adrenal-stimulating potency of the hormone.

On the other hand, modification of the amino acid residues within the NH_2-terminal sequence results in biological inactivation. By subjecting ACTH to the action of aminopeptidase, White (80) showed that 64% of the physiological activity was destroyed if more than one-half of both the first serine and the subsequent tyrosine were removed. Moreover, oxidation of the *N*-terminal serine residue with periodate (15, 23, 25) caused almost complete loss of ACTH activity (see Fig. 2). Acetylation of the α-NH_2 group in guanidinated ACTH also resulted in complete loss of activity (24). Recently, Waller and Dixon (76) were able to acetylate selectively the terminal amino group of ACTH and showed the resulting *N*-acetylated ACTH to possess less than 10% of the activity of the untreated hormone.

In 1955, Dedman *et al.* (11) showed that ACTH can be oxidized to a biologically inactive product by the action of hydrogen peroxide at pH 7–7.5 and at room temperature, and, further, that the inactivated hormone can be

restored to a potency equal to or even higher than that of the starting material by allowing it to react with cysteine hydrochloride at pH 1.9 and 80° C. for 18 hours. Thioglycolic acid was also reported to be effective in this respect but H_2S, ascorbic acid, and electrolytic reduction were not.

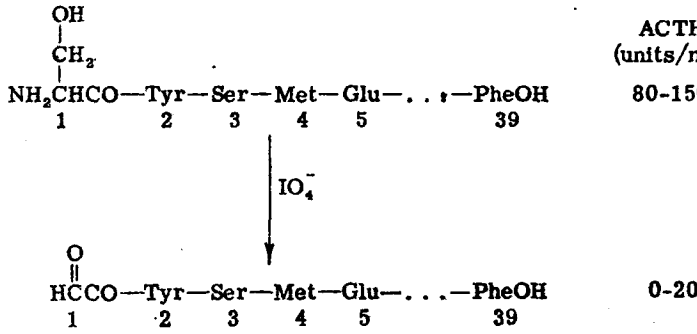

FIG. 2. Oxidation of ACTH with periodate.

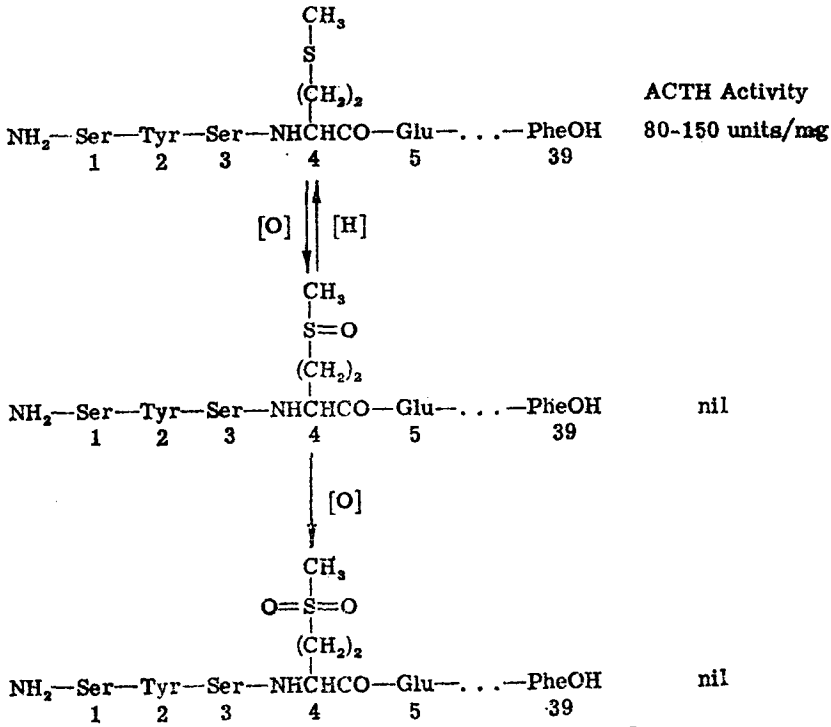

FIG. 3. Oxidation-reduction center of ACTH.

Dixon and Stack-Dunne (16) demonstrated that in the case of the H_2O_2-inactivated preparation of ACTH, a fall in retention volume occurs when the material is submitted to chromatography on the carboxylic ion-exchange resin Amberlite IRC-50; reactivation by cysteine was shown to be accompanied by a reversal of the change in retention volume (14). The mysterious reactive group in the hormone which is responsible for the inactivation-reactivation phenomenon has now been identified as the methionine residue in position 4 of the amino acid chain. It was found that mild oxidation of ACTH caused the conversion of methionine to methionine sulfoxide and that the methionine sulfoxide analog of the hormone contains but little activity (12). Extensive oxidation, however, led to the formation of methionine sulfone, and this oxidized product cannot be reduced to the original hormone (see Fig. 3).

From these data, it is clearly evident that ACTH activity must reside in the *N*-terminal amino acid portion of the 39-amino acid polypeptide chain. This expectation was very recently confirmed when a nonadecapeptide with a structure identical to the first 19 NH_2-terminal amino acids of the native ACTH was synthesized and the synthetic product was found to possess adrenal-stimulating activity (54).

III. Biological Properties of ACTH

1. *Adrenal-Stimulating Potency and* in Vivo *Properties*

The biological potency of ACTH peptides has been estimated to be in the range of 80–150 I.U. per milligram on the basis of the capacity of these peptides to deplete ascorbic acid in the adrenals of hypophysectomized rats. Forsham *et al.* (22) reported that when α_s-ACTH (the main component of sheep ACTH) was assayed in man by the criterion of steroidogenesis elicited by intravenous injections, an activity equivalent to 150 I.U. per milligram of the peptide was obtained. The adrenocorticotropic activity of α_p-ACTH (the main component of porcine ACTH) as determined by the ***in vitro*** corticoidogenesis assay procedure was found to be 95 U.S.P. units per milligram (26). Table I presents the specific activities of ovine, porcine, bovine, and human ACTH as estimated by the ***in vitro*** assay method of Saffran and Schally (61). The hormones from the pituitaries of these four species were isolated by the countercurrent distribution procedure recently described (17). From the data in Table I, it is evident that ACTH from ovine, porcine, and bovine pituitaries had identical potencies whereas the human hormone possessed a lower potency. Lee *et al.* (43) also reported an activity of 26 U.S.P. units per milligram for human ACTH. Although the amino acid composition of the human hormone is identical with bovine and ovine

ACTH (43, 56), it is possible that its amino acid sequence in a certain portion of polypeptide chain is different from the sequence in bovine and porcine ACTH peptides and that within this area of difference lies the explanation for the low biological potency of the human hormone.

TABLE I

ACTH Activity of Adrenocorticotropins from Various Species

Species	*In vitro* steroidogenesis (units/mg.)
Human	52
Porcine	150
Ovine	177
Bovine	140

As would be expected, the adrenal weight and histology of both normal and hypophysectomized rats is affected by ACTH. In a study on the effect of α_s-ACTH on intact male rats of the Long-Evans strain, a daily dose (suspended in beeswax-peanut oil) of 15 μg. of the hormone for 4 days resulted in an increase in adrenal weight of nearly 100%, as well as an 80% reduction of thymus weight (51). It was interesting that with smaller doses of the hormone, the involution of the thymus gland proved to be the more sensitive indicator of ACTH activity.

In studies with the hypophysectomized animal, male rats of the Long-Evans strain, operated on at 40 days of age, were injected intraperitoneally with graded doses of α_s-adrenocorticotropin in a beeswax-peanut oil suspension once daily for 4 days, beginning 4 days after hypophysectomy. It was found that a daily dose of 7.5 μg. in 4 days gave a 100% increment of adrenal weight over the control (51). A straight-line relationship was observed to exist between the weight of the adrenal glands and the logarithmic function of the daily dose. Reduction of thymus weight also resulted from the hormonal administration, as would be expected.

Microscopic changes, similar to those previously described for the adrenals of hypophysectomized rats under the stimulation of purified ACTH preparations, were seen in the adrenal glands following the administration of α_s-ACTH.

In vitro and ***in vivo*** studies of adrenal glands with α_b-ACTH (the main component of bovine ACTH) have yielded some important observations about the pattern of steroids secreted from the adrenals under the stimulation of the hormone. 17α-Hydroxy-Δ^5-pregnenolone was the steroid isolated from canine adrenal venous blood during stimulation by α_b-ACTH. This compound was not found in adrenal vein blood without exogenous ACTH, nor

could it be demonstrated in the aortic blood of the dog with intravenous ACTH (9). In studies with incubates of adrenals of the American bullfrog *Rana catesbeiana,* aldosterone was the most abundant steroid secreted under the stimulation of ACTH (8, 72). Corticosterone was the second major steroid found in these incubates, the ratio of aldosterone to corticosterone being 3.6 to 1. No cortisol could be detected, although previous studies had revealed only cortisol and corticosterone as the major adrenal steroids secreted by both mammals and cold-blooded animals.

It has been postulated for some time that there is a separate factor in the pituitary responsible for erythropoiesis (75). Recent studies (69, 73) showed that the so-called pituitary erythropoietic hormone is very likely ACTH. It was found that a daily dose of 10 μg. of α_s-ACTH administered for 14 days to hypophysectomized rats produced a significant increase in hemoglobin, hematocrit, and circulating red cell volume. Furthermore, Evans *et al.* (20) reported that α_s-ACTH restored normal heat production (oxygen consumption) in hypophysectomized rats.

Preparations of purified ACTH have been shown to have fat-mobilizing (adipokinetic) activity in fasted mice (44, 60). Rosenberg (60) had suggested that this activity might reside in a contaminant rather than being a function of the ACTH molecule. When pure sheep ACTH became available, the adipokinetic effects of the hormone were reinvestigated (51). It was found that as little as 0.5 μg. of α_s-ACTH elicits a highly significant increase in liver fat during a 7-hour fast. It was noted that chymotryptic digestion abolishes almost entirely both the adipokinetic and adrenal-stimulating activities of ACTH. Thus, there is little doubt that the adipokinetic activity is inherent in the adrenocorticotropin molecule.

The ketogenic activity of porcine ACTH was demonstrated in 24-hour fasted male rats under light Nembutal anesthesia by estimation of the increase in blood ketone levels 3½ hours after intraperitoneal injection of 0.02 mg. of the hormone (19). Moreover, the ketogenic activity may be reversibly inactivated by H_2O_2 and cysteine. It was concluded by the Engels that this activity is an intrinsic property of the ACTH peptide molecule.

It is well established that pure ACTH peptides (46, 66) possess melanophore-expanding activity as assayed in hypophysectomized *Rana pipiens,* with the change in the melanophore index in the intact web serving as the criterion of response. Indeed, chemical modification of α_s-ACTH can dissociate these two activities, because the structural requirements for melanophore-expanding and adrenal-stimulating activities in ACTH peptides are different. It was found that esterification of α_s-ACTH does not reduce its melanophore-stimulating activity but does diminish its adrenocorticotropic

potency (47). This means that the free carboxyl groups in adrenocorticotropin are essential for its ACTH activity but are not implicated in its intrinsic melanophore-stimulating effect.

2. In Vivo *Effects in the Absence of Adrenals*

Early reports (64, 65) have demonstrated that ACTH preparations increase the thymolytic action of cortisone in the absence of adrenals. Moreover, the same investigators (38) found that purified ACTH was capable of stimulating the preputial glands after adrenalectomy and gonadectomy. These observations probably represent the first indication of extra-adrenal effects on the part of adrenocorticotropin. Recent studies with ACTH peptides have clearly shown that the hormone can exert biological effects in rats whose adrenals have been removed. For example, Menkin (58) reported that α_s-ACTH directly suppresses the increased capillary permeability at the site of inflammation in rats without adrenal glands. In adrenalectomized rats maintained with cortisol, α_s-ACTH exerts an adipokinetic effect (51), although the response was not found to be as great as the normal animal. Marks *et al.* (57) reported that ACTH produces hypergranulation of the renal juxtaglomerular cells in the absence of both the pituitary and the adrenal glands. The metabolism of cortisol in adrenalectomized patients (13) and mice (4) has been shown to be altered by ACTH injections.

3. In Vitro *Properties*

By the *in vitro* frog skin assay procedure (68), it was estimated that α_s-ACTH possesses a melanocyte-stimulating activity (51) of approximately 1×10^8 units per gram, which amounts to 1/100 of the activity of α-MSH. This is clearly an extra-adrenal property of ACTH. In addition, the porcine hormone as described by the Armour investigators (29) has been shown to possess an MSH activity of 1.7×10^8 units per gram. Although this *in vitro* effect of ACTH in darkening frog skins serves as the basis for one of the standard bioassays for MSH activity, this is not the only *in vitro* effect of ACTH. White and Engel (79) reported that ACTH was highly effective in stimulating hydrolysis of neutral fat and release of nonesterified fatty acid when allowed to incubate with rat adipose tissue in plasma and other suitable media. It was suggested that the lipolytic response of isolated rat adipose tissue to ACTH is due to direct activation of a lipase in the tissue (36). Weinberg *et al.* (78) showed that the uptake and oxidation of glucose by rat mammary tissue *in vitro* is increased by ACTH. Very recently, Krahl and his co-workers (41) were able to demonstrate that ACTH inhibits the incorporation of amino acids into protein in rat adipose tissue.

TABLE II
Some Biological Properties of ACTH

Experimental condition	Biological effects of ACTH
In vivo	1. Increases the weight of adrenal glands
	2. Repairs the adrenal of hypophysectomized rats
	3. Promotes corticoid production as estimated in the adrenal venous blood
	4. Causes eosinopenia and thymic involution
	5. Enhances erythropoiesis in hypophysectomized animals
	6. Elevates metabolic rate of hypophysectomized rats
	7. Induces deciduoma in hypophysectomized-oophorectomized rats
	8. Increases weights of sex accessories of hypophysectomized-castrated rats
	9. Maintains muscle glycogen in hypophysectomized animals
	10. Provokes glycogen deposition in the liver
	11. Acts as a galactopoietic agent
	12. Exerts antagonistic action to growth hormone
	13. Causes an increase of liver fat in fasted animals
	14. Induces an elevation of serum-free nonesterified fatty acids
	15. Increases blood ketone bodies in fasted rats
	16. Stimulates melanophore expansion in amphibians and reptiles
In vivo in the absence of adrenals	17. Suppresses the increased capillary permeability induced by exudin
	18. Causes an increase in liver fat in animals maintained with corticoids
	19. Produces hypergranulation of the renal juxtaglomerular cells
	20. Influences metabolism of cortisol
In vitro	21. Stimulates corticosterone production in the rat adrenal and aldosterone production in the bullfrog adrenal
	22. Causes melanophore expansion in skins of amphibians and reptiles
	23. Releases nonesterified fatty acids from rat epididymal fat pad
	24. Induces the uptake and oxidation of glucose by rat mammary tissue
	25. Inhibits incorporation of amino acids into adipose-tissue protein

The variety of the biological effects of ACTH, briefly discussed above and summarized in Table II indicate clearly that the hormone does not act solely through the mediation of the adrenal cortex, but that it also exercises extra-adrenal functions. Thus, we can now see the misconception inherent in the name originally coined for the hormone from its major biological activity, i.e., adrenocorticotropic hormone. It was assumed for a long time that any ACTH preparation which can elicit a biological response *in vitro* or in adrenalectomized animals was contaminated with some active component(s) other than the ACTH itself. We now know that this is not true.

IV. Synthesis of Large Peptides Related to ACTH

Once the complete structure of a natural product is known, it is necessary to prove the structure by synthesis and to obtain a synthetic product with biological activity. In 1956, Boissonnas and his co-workers (7) published a preliminary note of the synthesis of the methyl ester of an icosapeptide corresponding to the first 20 *N*-terminal amino acids of the ACTH molecule, which exhibited an ACTH activity of 2–3 units per milligram as measured by the *in vitro* assay method (61). These investigators (7) stated that one explanation for the low activity might be "the possibility of a certain amount of racemization at certain steps of the synthesis." It is also possible that the low activity of the Boissonnas peptide is due to esterification of the COOH-terminal valine residue.

In 1958, this same group of Swiss chemists (6, 27) described for the first time the complete synthesis of α-MSH, a tridecapeptide (28) containing a sequence identical with the *N*-terminal portion of ACTH, except that the α-amino group is blocked with an acetyl residue and the *C*-terminus with an amide. The glutaminyl, formyllysyl, and other analogs of α-MSH have also been synthesized by a group of investigators at the University of Pittsburgh (30–33). Table III presents activities of synthetic α-MSH and its analogs as reported in the literature. It should be pointed out that the Pittsburgh investigators (31) have claimed that the deacetylated α-MSH "exhibited consistently an activity of < 0.1 I.U./mg." on the basis of ascorbic acid depletion and plasma corticosterone elevation tests for ACTH.

Since the publication in November, 1960, of the synthesis of a nonadecapeptide (54), a synthetic product whose sequence is identical with the first 19 NH_2-terminal amino acids of native ACTH and which possesses both ACTH and MSH activities, two other laboratories have announced synthetic ACTH polypeptides (34, 63). Kappeler and Schwyzer (39) have also just reported the synthesis of a product with a sequence identical with the first 24 amino acids of ACTH, but no activity was given (see Table IV).

TABLE III

Synthetic α-MSH and Its Analogs

R^2 R^3

(R^1-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Try-Gly-Lys-Pro-Val-NH_2)

1 2 3 4 5 6 7 8 9 10 11 12 13

Peptides	MSH Activity (units/gm.)	Laboratory	Date	Reference
$R^1 = CH_3CO$; $R^2 = OH$; $R^3 = H$ (α-MSH)	3.3×10^{10}	Sandoz	September, 1958	(6)
			June, 1959	(27)
$R^1 = H$; $R^2 = OH$; $R^3 = H$	1.9×10^{9}	University of Pittsburgh	May, 1961	(31)
$R^1 = CH_3CO$; $R^2 = NH_2$; $R^3 =$ tosyl	2×10^{9}		November, 1959	(32)
$R^1 = CH_3CO$; $R^2 = NH_2$; $R^3 =$ formyl	1 to 2 $\times 10^{10}$		July, 1960	(33)
$R^1 =$ carbobenzoxy; $R^2 = NH_2$; $R^3 =$ tosyl	0.8×10^{8}		December, 1958	(30)