The melanophore-stimulating activity of corticotropin

Most of the melanophore-stimulating (MSH) activity of pig pituitary extracts has been separated[1,2,3,4] from adrenocorticotropic (ACTH) (ascorbic-acid depleting[5]) activity. In unpublished experiments, RABEN, ASTWOOD AND ROSENBERG, together with STACK-DUNNE AND DIXON, found that on chromatography of oxycellulose concentrates of corticotropin[6,7], under conditions suitable for the isolation of corticotropin A_1[8], most of the MSH activity was unretarded, while some remained with the corticotropins. Corticotropin A_1 prepared by the method of DIXON AND STACK-DUNNE[8] possesses 10 I.U./mg MSH activity, in comparison with 1,500 I.U./mg of purified "melanophore-stimulating peptide"[4]*. The corticotropins isolated by BELL[9] also possess MSH activity.

This degree of MSH activity could be intrinsic to the corticotropin itself, as BELL[9] suggests, or it could be due to 0.5–1 % of contaminating melanophore-stimulating peptide. The discovery by DEDMAN, FARMER AND MORRIS[10] of conditions for reactivating peroxide-treated ACTH preparations, and the separation of active and inactivated corticotropins A_1 by DIXON AND STACK-DUNNE[8] provided means of studying this problem.

Corticotropin A_1, inactivated by peroxide treatment[8], was reactivated by treatment with a thiol[10], following the details of DIXON[11]. The result of assays of MSH activity in frogs** (one of which is shown in Table I), was that this activity had also been lost on peroxide treatment and regained on heating with a thiol.

TABLE I

MSH ACTIVITY OF PEROXIDE-TREATED AND REACTIVATED CORTICOTROPIN A_1

Corticotropin A_1 was treated with hydrogen peroxide (0.8 *M*) at pH 6.7 for 30 minutes and reactivated by treatment with thioglycollate (0.2 *M*) at pH 3.5 at 70–82° for 2.5 hours.

Injection (4 μg)	*Melanophore index*[13]		
	Before injection	*One hour after injection*	*Change*
None	1.5, 2.0	2.0, 1.7	+ 0.1
Peroxide-treated corticotropin A_1	1.5, 1.5	2.0, 1.5	+ 0.25
Peroxide-treated corticotropin A_1 heated with thioglycollate	1.5, 2.0	4.0, 3.5	+ 2.0

The MSH activity of purified melanophore-stimulating peptide[4] is similarly destroyed by peroxide and regenerated by heating with a thiol. One of the assays which showed this is given in Table II. The failure of potassium borohydride to regenerate the activity, also shown in Table II, was also found with the ACTH activity of corticotropin[8,10], and suggests that a similar chemical change may be involved in the inactivation and regeneration of both hormones.

TABLE II

EFFECTS OF TREATMENT WITH PEROXIDE, BOROHYDRIDE AND THIOGLYCOLLATE ON THE MSH ACTIVITY OF MELANOPHORE-STIMULATING PEPTIDE[4]

All injections of 8 mμg peptide after various treatments.

Treatment	*Melanophore index one hour after injection*
None	3.3, 2.9
KBH_4 (0.1 *M*, pH 8.5–9.5, 25 min)	3.2, 3.5
H_2O_2 (0.8 *M*, pH 6.7, 30 min)	1.3, 1.2
H_2O_2 (0.8 *M*, pH 6.7, 30 min) then KBH_4 (0.1 *M*, pH 8.5–9.5, 25 min)	1.5, 1.3
H_2O_2 (0.8 *M*, pH 6.7, 30 min) then thioglycollate (0.2 *M*, pH 3.5, 68–75°, 3.5 h)	2.5, 3.4
No injection	2.0, 1.4

* I am most grateful to Professor LANDGREBE for these assays. In my own hands 1 μg corticotropin A_1 gives the same order of response in MSH assays as 8 mμg purified peptide[4].

** Assay modified from that of LANDGREBE AND WARING[12]: injections of 0.4 ml were used.

A sample of melanophore-stimulating peptide[4] was applied to a column of a carboxylic ion-exchange resin under conditions in which corticotropins A_1 and A_2 are retarded[8], and its MSH activity was recovered in the unretarded fraction, while the fraction of column effluent which would have contained corticotropin A_1 was inactive at the same dose level. It may therefore be this factor which explains the unretarded MSH activity observed by ASTWOOD, DIXON, RABEN, ROSENBERG AND STACK-DUNNE (unpublished) on chromatography of ACTH concentrates. The heterogeneous protein unretarded by the chromatographic columns in the preparation of corticotropin A_1[8], may be obtained in the dry state by precipitation as the picrate and subsequent removal of picrate by ion exchange[6]. This material possesses a higher MSH potency than corticotropin A_1 and probably contains much of the melanophore-stimulating peptide of the pituitaries.

Samples of peroxide-treated corticotropin A_1 were treated at pH 3.5 with 0.2 *M* thioglycollate at 70° for 2 hours. This short treatment gave only partial regeneration of corticotropin A_1, as shown by chromatography (Fig. 1). MSH assays (specimen assays are given in Table III) on two such chromatograms showed that the faster peak was inactive and the slower peak active at 1 μg doses. The slower peak also possessed the ACTH activity while the faster peak was inactive, as shown by an ascorbic-acid depletion test[5].

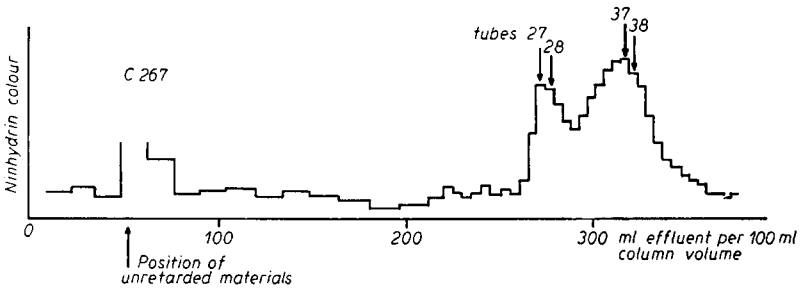

Fig. 1. Chromatogram of partially reactivated peroxide-treated corticotropin A. MSH assay (Table III) showed tube t27 inactive, t28 slightly active and t37, 38 active at 1 μg doses. Peroxide-treated corticotropin A_1 (10.9 mg) was dissolved in the phosphate buffer used for chromatography[8] (pH 6.7, 0.2 *M* phosphate) (1.20 ml). To 1.00 ml thioglycollic acid (0.017 ml) was added (final concentration 0.2 *M*, pH 3.5) and the solution was heated to 71–75° for 2 hours. After cooling NaOH solution (0.667 ml 0.3 *N*) and phosphate buffer (0.333 ml) were added and the solution chromatographed on a column of finely ground Amberlite IRC-50 of 42.9 cm × 0.68 cm².

TABLE III

ASSAYS OF COLUMN EFFLUENT FROM A CHROMATOGRAM OF PEROXIDE TREATED CORTICOTROPIN A_1 WHICH HAD BEEN PARTIALLY REGENERATED (Fig. 1)

The original solution was tested at a dose of 1 μg. The tubes were tested at the same dose with respect to ninhydrin colour.

Injection	*Melanophore index*		
	Before injection	*One hour after injection*	*Change*
Assay 1			
None	2.0, 2.0	1.5, 1.7	—0.4
Tube t27 (fast peak)	2.3, 2.3	2.5, 2.8	+0.35
Tube t37 (slow peak)	1.3, 2.3	2.7, 3.3	+1.2
Original solution (before thiol treatment)	2.5, 2.3	2.8, 1.5	—0.25
Assay 2			
None	1.5, 1.8	1.3, 1.8	—0.1
Tube t27 (fast peak)	1.5, 1.5	1.5, 1.7	+0.1
Tube t28 (fast peak)	2.0, 1.7	2.4, 3.0	+0.85
Tube t38 (slow peak)	1.7, 2.3	4.5, 3.8	+2.15

A substance isolated by ion-exchange chromatography could perhaps be contaminated with a substance which is unretarded in the chromatographic system, since a complex between the two substances might be formed. The ease of separation of most of the MSH activity from the corticotropin and the constant amount of residual activity[9] provided some evidence that this residual activity was intrinsic. The fact that MSH activity was lost on treatment with peroxide and regained on heating with a thiol, together with the ACTH activity[10], and together with a physico-chemical change (the retention volume on chromatography)[8,11], provided further evidence that the activity was a property of corticotropin itself, but this argument was weakened by the similar behaviour of the activity of purified melanophore-stimulating peptide[4].

The chromatographic separation of two substances from partially regenerated corticotropin A_1, one possessing both ACTH and MSH activities and one inactive in both tests, provides strong evidence that both activities are intrinsic to one molecule. Both peaks would probably be active if the activities were due to a contaminant which formed a complex stable under the conditions of chromatography. The simplest explanation of the results is that whenever a molecule of the faster-running material is converted into the slower-running it regains both activities, although more complex explanations can be proposed. Thus corticotropin appears to possess intrinsic MSH activity although it has less than 1 % of the potency of the melanophore-stimulating peptide.

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Bombixin, a sex attractant discharged by female moth, *Bombix mori*

Immediately after emergence both the male and the female of *Bombix mori* copulate. The female moth takes up a characteristic calling position as if she would discharge some kind of scent from her hip in order to attract the male insect.

The male, when coming near the female, seems to feel excitement, flapping the wings exquisitely and lifting her abdominal hip. From a distance of 10 cm the male gradually approaches the female vibrating his wings violently and from a distance of 5 cm he goes more or less straight to her. Throughout this approach the female lifts her hip, putting out a tongue-like body from the end of the abdomen and seems to discharge some gaseous substance. The male takes a copulating attitude so long as the female scent substance is in the air, even in the absence of the female moth. This sexual scent seems very specific and showed no relation to ordinary odiferous substances. We call this sex attractant "bombixin".

In order to obtain bombixin as pure as possible, we treated hips of sixty thousand female moths after copulation (we think it is better to use virgin moths) with alcohol in a mortar by the addition of a small amount of quartz sand. After standing overnight the mixture was filtered. The filtrate which amounted to 15 l was evaporated under the reduced pressure to 2.2 l and saponificated by the addition of 600 grams of solid caustic soda at a temperature not exceeding