The Action of Interstitial Cell-Stimulating Hormone upon Tyrosinase Activity in the Weaver Bird (Steganura paradisaea)*

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Interstitial cell-stimulating hormone (ICSH)† has been shown to cause pigmentation of the feathers of weaver birds (Witschi, 1940). The black color resulting from exogenous administration of ICSH is due to the deposition of melanin in the regenerating feathers (Rawles, 1960). It is probable that the rate-limiting step in the biosynthesis of melanin is the conversion of tyrosine to 3,4-dihydroxyphenylalanine (dopa) catalysed by the enzyme tyrosinase (Lerner, 1953). This report demonstrates that ICSH administered to weaver birds in vivo increases the activity of tyrosinase in the skin from which responding feathers arise (the so-called right and left ventral feather tracts).

Methods and Materials Weaver birds (Steganura paradisaea) were imported from Africa and cared for as described elsewhere (Hall et al., 1965). The ventral feather tracts were plucked and 7 days later one feather tract was removed for measurement of tyrosinase activity. The bird was then injected with ICSH (500

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†Abbreviations used - FSH: follicle-stimulating hormone. ICSH: interstitial cell-stimulating hormone (also called luteinizing hormone). MSH: melanocyte-stimulating hormone.

µg dissolved in saline, intramuscularly). Twenty four hours later the bird was sacrificed and measurement of tyrosinase activity performed on the remaining feather tract. In this way each bird acted as its own control.

Tyrosinase was measured by the method of Pomerantz (1964). Feather tracts (approximately 200 mg each) were homogenized in potassium phosphate buffer (0.1M pH 6.8) and centrifuged at 700 x g for 10 minutes at 0°. The supernatant fraction (approximately 2 mg protein per feather tract) was then incubated for 1 hour at 37° with the following additions: L-tyrosine -3,5-^{3}H (5 µc:0.1 µmole per flask), DL-dopa (0.01 µmole per flask) and phosphate buffer to a final volume of 2 ml at pH 6.8. Tritiated water was recovered by passing the incubation medium through a charcoal-celite column and tritium content measured as described by Pomerantz (1964). Enzyme activity is expressed as mµmoles of tyrosine hydroxylated per hour per mg protein. Non-enzymatic exchange between tyrosine-3,5-^{3}H and water was measured by incubation with enzyme which had been boiled for 10 minutes and the value subtracted from the tritium content of water in the presence of untreated enzyme. The non-enzymatic exchange was equivalent to 0.5 mµmole ± 0.01 (SD) of tyrosine-^{3}H per hour per flask.

In order to demonstrate that the exchange of tritium between tyrosine -3,5-^{3}H and water measured in these studies was due to tyrosinase, measurements of dopa-^{3}H were made when incubation was performed in the presence of ascorbate to prevent oxidation of dopa-^{3}H (Pomerantz, 1964). Half of the tritium of tyrosine-3,5-^{3}H exchanges with water and half appears in dopa-^{3}H (Pomerantz, 1964).

Because the tritiated compounds undergo some exchange with water, it was convenient to use tyrosine-u-^{14}C in order to prepare dopa-^{14}C for determination of radio-chemical purity. Apart from the use of tyrosine-u-^{14}C (1.0 mole; 10^6 cpm/flask) incubation conditions were the same as those mentioned above. Dopa-^{14}C was isolated by chromatography on aluminum oxide (Pomerantz, 1964).

L-tyrosine-3,5-^{3}H (Batch 4) was purchased from Nuclear Chicago and L-tyrosine-u-^{14}C (lot No 69-162A-19) from New England Nuclear Corporation. Ovine ICSH and FSH were gifts of the Endocrine Study Section of the National Institutes of Health (NIH-LH-S-7 and -S-8 and NIH-FSH-2). Tyrosine decarboxylase from S. faecalis was obtained from Worthington Biochemical Corporation. Synthetic α-MSH was generously provided by CIBA.

Results The stoichiometry of the tyrosinase assay was shown by comparison of the tritium content of water with that of dopa-^{3}H in the same assay on six different batches of enzyme (Table 1).

Table 1

The Stoichiometry of Tyrosinase Assay on Feather Tract

Experiment	^{3}H in Water (dpm)	Dopa-^{3}H (dpm)	Dopa-^{3}H relative to ^{3}H in water as 100
1	14,100	12,100	86
2	43,000	42,000	98
3	27,200	28,000	104
4	19,000	18,000	95
5	21,000	23,000	110
6	16,000	14,800	93

The conditions of incubation are those reported by Pomerantz (1964). Each experiment was performed on one bird previously treated with ICSH (three daily injections, 100 µg each).

It will be seen that the tritium content in dopa-^{3}H is approx-

imately equal to that in water, indicating that measurement of tritium in the water eluted from the charcoal-celite column is a valid measure of tyrosinase activity. Table 2 provides evidence

Table 2

Recrystallization of Dopa-^{14}C

Stage of Purification	Specific Activity of Dopa-^{14}C (dpm/mg)	
	Sample A	Sample B
After addition of carrier	614	912
1st recrystallization	603	914
2nd "	606	908
3rd "	596	914
4th "	608	916

Dopa-^{14}C was recrystallized from water after addition of authentic dopa (10 mg). A drop of N hydrochloric acid was added during recrystallization in order to prevent oxidation of dopa.

for the radiochemical purity of dopa-^{14}C isolated after incubation of homogenate of feather tract with tyrosine-u-C^{14} and ascorbate (Pomerantz, 1964). It will be seen that within the limits of experimental error, the specific activity of two samples of dopa-^{14}C remained constant through repeated crystallization. Samples A and B (Table 2) were obtained from enzyme preparations prepared from different birds. Further evidence for the identity of dopa-^{14}C was provided by treating a sample with tyrosine decarboxylase from S. faecalis. The specific activity of dopa-^{14}C was 3,182 dpm/m mole and that of the dopamine-^{14}C following decarboxylation and purification by paper chromatography on t-butanol/formic acid/water 70:15:15, was 3,069 dpm/m mole, after correcting for the loss of $^{14}CO_2$ incurred during the conversion of dopa to dopamine. Dopamine-^{14}C behaved like the authentic compound in this system of chromatography. It is therefore concluded that the assays

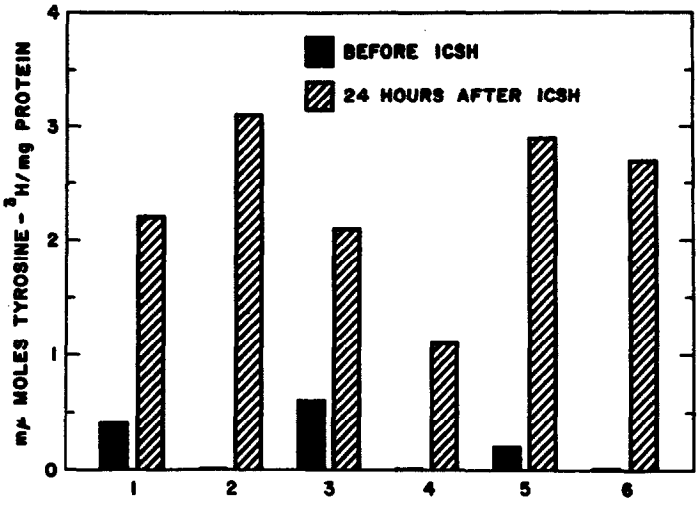

Figure 1. The effect of ICSH upon tyrosinase activity in feather tracts of S. paradisaea. The conditions of assay are given in the text. Each pair of bars represents a single determination of tyrosinase activity on each of the two feather tracts of one bird.

reported here provide a valid measure of tyrosinase activity.

Figure 1 shows the results of 6 experiments in which tyrosinase activity in feather tracts of S. paradisaea was measured before and 24 hours after a single intramuscular injection of ICSH (500 μg). It will be seen that activity of the untreated feather tract varies considerably and in some cases no activity could be measured (ie < 0.01 mumole tyrosine hydroxylated per hour per mg protein). In each bird, however, considerable increase was observed following injection of ICSH. Experiments using other hormones and saline are shown in Table 3. Neither MSH, ACTH, FSH nor saline produced demonstrable increase in tyrosinase activity.

Discussion The method of assay for tyrosinase described by Pomerantz (1964) is capable of measuring small amounts of the enzyme in skin. The findings reported here indicate the presence of tyrosinase in the feather tracts of S. paradisaea. The level of enzyme activity in untreated birds is low and variable but

considerable increase is observed within 24 hours of a single injection of ICSH (Figure 1). This observation is in keeping with the fact that a single injection of ICSH causes the appearance of a black bar on feathers regenerating at the time of injection; this action of the hormone has been shown to be specific for ICSH (Segal, 1957). It is of interest to note that ACTH increases tyrosinase activity in fish (Chavin et al., 1963) and that ACTH and MSH have been shown to cause darkening of pelage in the weasel

Table 3

Tyrosinase Activity of Feather Tracts before and after Injection of Various Hormones

Hormone	Dose	Tyrosinase Activity (mμmoles/mg protein/hour)	
		Before Treatment	After Treatment
α MSH	100 μg	0.1 <0.01 <0.01	0.1 <0.01 <0.01
FSH	100 μg	0.1 0.2 <0.01	<0.01 0.2 <0.01
ACTH	2 units	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
Saline	0.1 ml	0.1 <0.01 <0.01	0.2 <0.01 <0.01
ICSH	100 μg	0.1 <0.01	0.8 0.4

The effect of various hormones and of saline upon the tyrosinase activity of feather tracts of S. paradisaea. The hormones (dissolved in saline) and saline were injected intramuscularly and tyrosinase assays were performed before and 24 hours after injection.

(Rust, 1965), both of these hormones were without demonstrable effect on tyrosinase activity in S. paradisaea. MSH and ACTH do not cause the appearance of black bars on regenerating feathers of these birds (Witschi, 1955; Segal, 1957).

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