The Action of Interstitial Cell Stimulating Hormone upon Avian Tyrosinase*

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ABSTRACT: Interstitial cell stimulating hormone (ICSH) is known to cause blackening of feathers in weaver birds (including *Steganura paradisaea*), owing to the deposition of melanin in the feathers. It is believed that ICSH is responsible for the black plumage seen in male weaver birds during the breeding season. The present studies demonstrate that ICSH administered *in vivo* increases the tyrosinase activity of the skin from which feathers grow (feather tracts). The properties of the enzyme are consistent with those of tyrosinase from other sources and the exact nature of the enzymatic activity measured is demonstrated. The response of feather-tract tyrosinase to ICSH is specific for this hormone and is shown to be confined to the region of skin from which responding feathers grow. The conditions necessary for ICSH to stimulate tyrosinase activity and the specificity of this action are similar to those described for the change in feather color produced by this hormone. It is, therefore, concluded that the action of ICSH in causing the deposition of melanin in feathers is, at least in part, attributable to increased tyrosinase activity.

During the breeding season male weaver birds (including *Steganura paradisaea*) wear a colorful nuptial plumage in which most of the feathers are black (Witschi, 1961). Between breeding seasons male and female birds are indistinguishable with respect to plumage color—both sexes are grayish brown (Witschi, 1961). Black bars appear on the feathers of birds injected with interstitial cell stimulating hormone (ICSH),[1] during the period of feather regeneration which follows plucking (Witschi, 1961). This response to ICSH occurs in birds of either sex and is taken to mean that ICSH plays an important role in the development of nuptial plumage in the male bird. This response is specific for ICSH (Segal, 1957) and represents an extragonadal action of the hormone since black bars appear in regenerating feathers when ICSH is administered to castrate birds of either sex (Witschi, 1961).[2] It appears likely on histological grounds that the black color of the feathers is due to the deposition of melanin (Witschi, 1940).

It is generally believed that the slow or rate-limiting step in the biosynthesis of melanin is the conversion of tyrosine to 3,4-dihydroxyphenylalanine (dopa); this reaction is catalyzed by the enzyme tyrosinase (EC 1.10.31, *O*-diphenol:O_2 oxidoreductase) (Lerner, 1953). The present studies were undertaken to determine whether ICSH is capable of increasing tyrosinase activity in the skin from which responding feathers grow (the so-called feather tracts). Some of the findings reported here were presented in preliminary form (Okazaki and Hall, 1965).

Experimental Section

Birds. Steganura paradisaea[3] were imported from Africa and kept as described elsewhere (Hall *et al.*, 1965). Before use in the following experiments, birds were plucked on the ventral surface in such a manner as to expose the right and left ventral feather tracts (ventral pterylae) from which feathers regenerate following plucking. The white tips of regenerating feathers emerge 7–10 days after plucking. At various stages after plucking, one ventral feather tract was removed under light ether anesthesia, the skin was sutured, and the bird was injected with ICSH 100 μg (or other substance to be tested) intramuscularly in saline. Tyrosinase was measured in the feather tract. Twenty-four hours later birds were sacrificed and tyrosinase activity was measured in the remaining feather tract of each bird. Birds were plucked at 4 PM and surgical procedures performed between 9 AM and 11 AM. The number of days after plucking is expressed here in such

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[1] Abbreviations used: ICSH, interstitial cell stimulating hormone; ACTH, adrenocorticotrophin; dopa, β-(3,4-dihydroxyphenyl)-L-alanine; dopamine, β-(3,4-dihydroxyphenyl)ethylamine; FSH, follicle-stimulating hormone; MSH, melanocyte-stimulating hormone; TSH, thyroid-stimulating hormone; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-2-(5-phenyloxazolyl)benzene.

[2] For further details of this and other aspects of the complex factors involved in the regulation of feather color, the reader is referred to Witschi (1961).

[3] The authors are grateful to Dr. Kenneth C. Parkes, curator of birds, Carnegie Museum, Pittsburgh, Pa. 15213, who identified the birds used in the present studies.

a way that the first day begins 24 hr after feathers were plucked.

Tyrosinase Assay. The method of measuring tyrosinase activity in feather tracts is that reported by Pomerantz (1964). Feather tracts were homogenized in potassium phosphate buffer (0.1 M, pH 7.8) and centrifuged at 700*g* for 10 min; the supernatant fraction was used in these experiments. The supernate was kept on ice before incubation with tyrosine-3,5[^{3}H] for 1 hr at 38°. Hydroxylation of tyrosine-3,5[^{3}H] causes exchange of one tritium atom with water; the second tritium atom gives rise to dopa[^{3}H]. The assay is based upon measuring the tritium content of water after removing unused tyrosine[^{3}H] by column chromatography (Pomerantz, 1964; Okazaki and Hall, 1965). Nonenzymatic exchange of tritium between tyrosine[^{3}H] and water was measured by incubating heated enzyme under the same conditions as those used for untreated enzyme. The value for nonenzymatic exchange was substracted from the value for untreated enzyme. Enzymatic activity is expressed as mμmoles of tyrosine hydroxylated/mg of protein per hr.

The expression standard conditions refers to incubation in phosphate buffer 0.1 M pH 7.8 for 1 hr at 38° with tyrosine 5 μc, 0.1 μmole/flask, and DL-dopa, 0.01 μmole/flask, in a final volume of 2 ml at pH 7.8. One feather tract contains approximately 2 mg of protein and the nonenzymatic exchange of tritium was equivalent to 0.5 ± 0.06 (std dev) mμmole in 20 determinations. Under the conditions reported, zero activity means <0.01 mμmole of tyrosine hydroxylated/mg of protein per hr. Statistical analysis of tyrosinase activity was based upon paired t test.

ICSH in vitro. Two methods were used to determine whether ICSH affects tyrosinase activity when the hormone was added to feather tracts *in vitro*. In some experiments, slices of feather tract were prepared with scissors and added to the flasks containing tyrosine[^{3}H], dopa, and buffer as described above. At the end of 1 hr the skin was homogenized, incubation continued for a second hour, and the tritium content of water measured. In other experiments slices of skin were preincubated with and without ICSH and at the end of incubation were homogenized in phosphate buffer and incubated with tyrosine[^{3}H] under standard conditions.

Control Studies. Hormones other than ICSH and saline were tested exactly as described for ICSH. In one control experiment, six birds were injected with saline and six with ICSH (100 μg in saline intramuscularly) on the 7th day after plucking. Twenty-four hours after injection the birds were sacrificed and tyrosinase activity was measured in feather tracts, liver, and the skin from the region between the tracts from which feathers do not grow. The preparation of the tissue and conditions of assay were those described above.

Identification of Tyrosinase Activity. In order to demonstrate that the activity measured was the result of tyrosinase activity two lines of evidence were obtained: the stoichiometry of the tritium exchange was demonstrated and the identity of the product of the reaction (radioactive dopa) was established.

STOICHIOMETRY OF TRITIUM EXCHANGE. In order to show that the tritium content of dopa was equal to that of water, samples of enzyme prepared from birds pretreated with ICSH were incubated under standard conditions except that 6 μmoles of neutralized ascorbate was added to prevent oxidation of dopa (Pomerantz, 1964). Following incubation, one-half of the incubation medium was used to determine the tritium content of water resulting from enzymatic activity (see above) and the remainder used to determine the tritium content of dopa. To the latter half 400 μg of dopa was added and unchanged tyrosine[^{3}H] was removed by applying to a column of 3 g of aluminum oxide. The column of aluminum oxide was eluted with ammonium acetate (0.5 M) 30 ml, (tyrosine fraction) water 30 ml, and acetic acid (0.5 M) 30 ml. The acetic acid fraction was purified by chromatography a second time in the same system. Aliquots of the dopa fraction (acetic acid), were taken for the estimation of dopa by a colorimetric method (Arnow, 1937) and for determination of tritium content by liquid scintillation spectrometry. From the recovery of added dopa (54–62% in 10 estimations), the tritium content of dopa[^{3}H] was determined.

IDENTITY OF DOPA[^{14}C]. In order to identify the product of the reaction it was more convenient to use ^{14}C than tritium. Samples of the enzyme prepared from birds pretreated with ICSH were incubated with tyrosine-U[^{14}C] and ascorbate. After incubation dopa[^{14}C] was isolated and purified by column chromatography (see above). Samples of dopa[^{14}C] prepared in this manner were either recrystallized from ethanol–hydrochloric acid or incubated with tyrosine decarboxylase (which also decarboxylates dopa, Brennerman and Kaufman, 1964). After each recrystallization of the former samples, specific activity of dopa[^{14}C] was measured by means of a colorimetric assay for dopa (Arnow, 1937) and by liquid scintillation spectrometry. Other samples were incubated with tyrosine decarboxylase 50 μg, pyridoxal phosphate 10^{-7} M in phosphate buffer 0.07 M and pH 6.8 in a final volume of 1 ml. After incubation the sample was lyophilized and applied to paper chromatograms in phenol–hydrochloric acid (100 g : 15 ml 0.1 N) (system A) for 20 hr. Dry chromatograms were examined by means of a strip scanner in order to locate dopa[^{14}C] and dopamine[^{14}C] which were identified by comparison with the running times of the authentic compounds. The specific activities of the two compounds were measured by fluorimetric assay for mass (Udenfriend, 1962) and liquid scintillation spectrometry for radioactivity.

Purification of Substrates. TYROSINE-3,5-[^{3}H]. Samples of tyrosine[^{3}H] were applied to paper chromatograms developed in system A (see above). Dry chromatograms were examined by means of a strip scanner which revealed the presence of a radioactive contaminant which contained less than 5% of the radioactivity of the sample. Tyrosine[^{3}H] eluted from the chromatograms and purified by column chromatography on aluminum oxide (see above) behaved as a single compound in paper chromatograms developed in both system A and 1-butanol–acetic acid–water (18:2:5, v/v)

(system B). When tyrosine-3,5-[^{3}H] purified by paper chromatography was compared with tyrosine-3,5-[^{3}H] not purified in this manner as substrate for tyrosinase assay, no significant difference was observed. Accordingly, preliminary purification was not performed routinely but new batches of tyrosine-3,5-[^{3}H] were examined as described.

TYROSINE-U[^{14}C]. When tyrosine-U[^{14}C] was examined as described above for tyrosine[^{3}H] a radioactive contaminant behaving like dopa[^{14}C] was observed; this contaminant accounted for as much as 10% of the total radioactivity in the sample. Before use in the present studies tyrosine-U[^{14}C] was purified by paper chromatography in system A to remove this contaminant. After such purification the tyrosine[^{14}C] behaved as a single compound when examined as described above for tyrosine[^{3}H].

Liquid Scintillation Spectrometry. Aliquots (0.5 ml) were counted in a Packard Tricarb liquid scintillation spectrometer (Model 315) after addition of 15 ml of scintillation fluid prepared as follows: naphthalene 100 g, 2,5-diphenyloxazole (PPO) 7 g, 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP) 0.3 g, Cab-o-sil 40 g, and dioxane to 1 l. Sufficient counts were allowed to accumulate to give a probable error of less than 5%. Addition of known amounts of radioactivity to samples revealed that significant quenching did not occur. The expression disintegrations per minute used here indicates that measurements of radioactivity were corrected for the prevailing efficiency of counting (31–34% for tritium and 52–56% for ^{14}C). Protein was determined by the method of Folin-Ciocalteau (Layne, 1962).

Materials. L-Tyrosine-3,5-[^{3}H] (batch no. 4) was obtained from Nuclear Chicago Corp. Tyrosine-U[^{14}C] (lot no. 69-162A-19) was purchased from New England Nuclear Corp. Materials for liquid scintillation spectrometry were of scintillation grade and were obtained from Packard Instrument Co., Inc. except Cab-o-sil which was purchased from Godfrey L. Cabot Inc., Boston, Mass. Interstitial cell stimulating hormone (NIH-LH-S-8), ovine FSH (NIH-FSH-S-2), bovine growth hormone (NIH-GH-B-9), bovine prolactin (NIH-P-B-1), and bovine TSH (NIH-TSH-B-1) were gifts from the Endocrine Study Section of the National Institutes of Health. Aluminum oxide was purchased from Merck and Celite (535) from Johns Manville. Deactivated ICSH prepared as described by Reichert (1961) was generously provided by Dr. L. S. Reichert, Jr., Emory University, Atlanta, Ga. This preparation (100 μg/flask) failed to stimulate slices of rabbit testis in a system which responded to less than 0.1 μg of untreated ICSH (Hall and Eik-Nes, 1962). Tyrosine decarboxylase was obtained from Worthington Biochemical Corp. and pyridoxal phosphate from Sigma Chemical Corp. The synthetic α-MSH used was generously donated by Ciba, ACTH was USP reference standard, and 2,4-dihydroxy-5,6,7,8-tetrahydropteridine was obtained from Calbiochem.

TABLE I: Stoichiometry of Tritium Exchange Resulting from Tyrosinase Activity.[a]

Sample	Tritium Content (dpm)		Dopa[^{3}H] Relative to TOH
	Dopa	TOH	
1	31,000	29,000	1.07
2	24,000	23,000	1.04
3	36,000	39,000	0.92
4	16,000	14,000	1.14

[a] Enzyme was prepared by injecting two birds for each sample with 100 μg of ICSH intramuscularly in saline daily for 3 days. The first injection was given 4 days after plucking and the birds were sacrificed 24 hr after the third injection. The method of measuring dopa[^{3}H] is described under Experimental Section.

Results

Nature of Enzyme Activity. In confirmation of an earlier report (Okazaki and Hall, 1965) the evidence presented demonstrates that feather tracts of the weaver bird contain tyrosinase. The stoichiometry of the tritium exchange which forms the basis of the assay used, expressed as dopa[^{3}H] formed *vs.* TOH resulting from enzyme activity, was shown to be 1:1 within the limits of experimental error (Table I). Moreover, the nature of the product of enzymatic activity (radioactively labeled dopa) was revealed by recrystallization to constant specific activity after addition of dopa (Table II) and by enzymatic conversion to dopamine (Table III). It will be seen that the specific activity of dopamine[^{14}C] is approximately equal to that of dopa[^{14}C] when allowance is made for the loss of the carboxyl ^{14}C.

TABLE II: Recrystallization of Dopa[^{14}C].[a]

Recrystallization	Specific Activity (dpm/mmole)
1st	612
2nd	601
3rd	618
4th	614

[a] Dopa[^{14}C] was prepared and specific activities determined as described in Experimental Section from three birds pretreated with ICSH (see legend to Table I).

Properties of Tyrosinase. Figures 1–4 show certain properties of the tyrosinase of feather tracts. Figure 1 shows the stimulating influence of dopa upon the enzymatic activity. In contrast to this stimulation, 2,4-

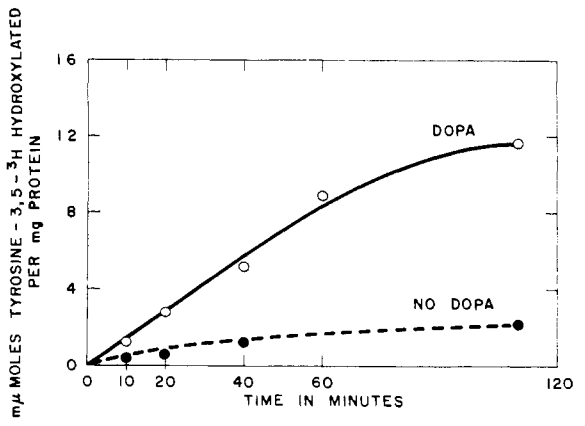

FIGURE 1: Enzyme was prepared from the feather tracts of six birds pretreated with ICSH as described beneath Table I. Incubation was performed for the times shown with and without dopa.

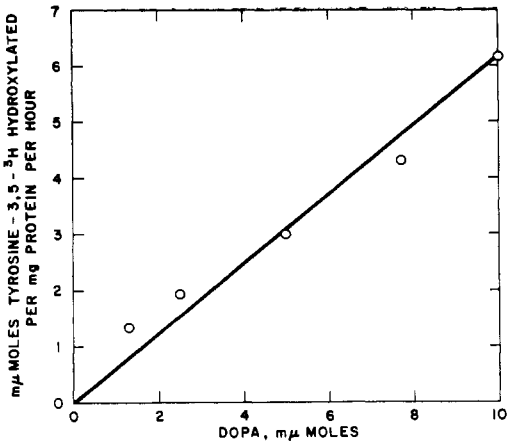

FIGURE 2: The influence of dopa on avian tyrosinase activity. Enzyme was prepared from five birds pretreated with ICSH (see legend to Figure 1).

TABLE III: Conversion of Dopa[^{14}C] to Dopamine[^{14}C].[a]

Sample	Specific Activity (dpm/mmole)	
	Dopa[^{14}C]	Dopamine[^{14}C]
A	1810	1760
B	2390	2270

[a] Samples of dopa[^{14}C] were prepared from birds pretreated with ICSH as described beneath Table I. The isolation of dopa[^{14}C] and the method of enzymatic conversion to dopamine[^{14}C] are described under Experimental Section. The specific activity of dopamine-[^{14}C] has been corrected for the loss of the carboxyl ^{14}C on the basis of uniform labeling.

dihydroxy-5,6,7,8-tetrahydropteridine in concentrations of 10^{-6}–10^{-3} M was without demonstrable effect on 12 separate preparations of avian tyrosinase (data not shown); each of these 12 preparations showed the usual response to addition of dopa. Figure 2 shows the effect of various concentrations of dopa upon the enzyme in the range 1–10 mμmole/flask; the reason for the apparent linearity of this response to dopa remains unexplained.

Figure 3 shows that the enzymatic reaction is maximal at 38° and Figure 4 that avian tyrosinase is most active at pH 7.8.

ICSH in vivo. Figure 5 shows the results of a series of studies in which tyrosinase activity of feather tracts was measured before and after a single intramuscular injection of ICSH (100 μg) at various stages after plucking the feathers from these tracts. Six birds were studied at each stage and a single determination of tyrosinase activity performed on each feather tract—one before and the second 24 hr after injection (see Experimental Section).

It will be seen that 1 day after plucking, ICSH was without demonstrable effect upon tyrosinase activity ($p > 0.2$). Three days after plucking enzyme activity was significantly greater after injection than before ($p < 0.01$). A more pronounced increase following ICSH is to be seen in birds examined 5, 7, and 9 days after plucking ($p < 0.001$ in each case). It will also be seen that in some birds no demonstrable activity was found before injection (*i.e.*, <0.01 mμmole of tyrosine hydroxylated/mg of protein per hr). A number of similar experiments performed with birds 7 and 9 days after plucking revealed that ICSH was without demonstrable effect 4 and 12 hr after a single intramuscular injection of the hormone (data not shown).

Addition of ICSH in vitro. In experiments not reported here, ICSH was without demonstrable effect when added *in vitro* to slices of feather tracts. After incubation with ICSH slices were homogenized and tyrosinase was assayed under standard conditions. In other experiments slices of feather tract were incubated with ICSH tyrosine[^{3}H] and dopa; the enzyme was assayed by homogenizing the tissue and continuing incubation without further change for 1 hr. In both types of experiment a wide variety of experimental conditions, including concentration of ICSH and duration of incubation, were tested without revealing any influence of ICSH *in vitro* upon the activity of feather tract tyrosinase.

Control Studies. Table IV shows that saline and a number of hormones including deactivated ICSH, FSH, MSH, and ACTH were without effect upon tyrosinase activity.

Table V shows that in six birds in which a response of feather tracts to ICSH 7 days after plucking was observed, no tyrosinase activity was detected in skin taken from the region between the feather tracts in either treated or control birds. The activity observed in liver was also not significantly influenced by ICSH ($p > 0.7$), although the nature of the tritium exchange measured

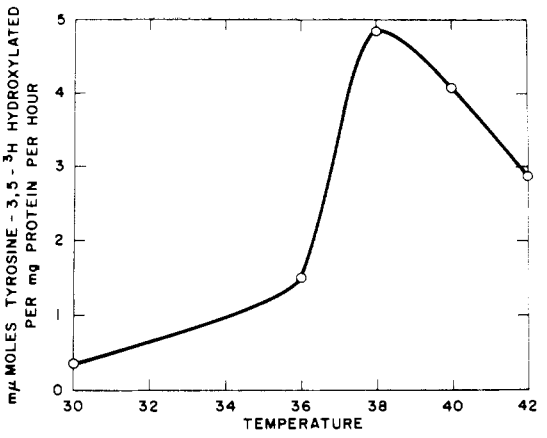

FIGURE 3: The influence of temperature of incubation upon tyrosinase activity.

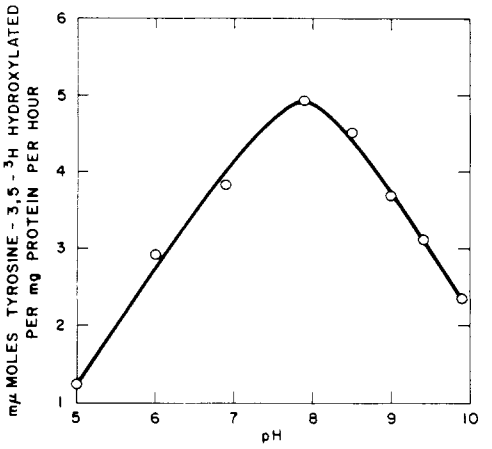

FIGURE 4: The influence of pH upon tyrosinase activity. Incubation was performed under standard conditions (see Experimental Section) except that the pH of phosphate buffer was varied as shown.

TABLE IV: Tyrosinase Activity before and after Administration of Saline and Hormones.[a]

Substance Administered	Tyrosinase Activity (mμmoles hydroxylated/mg of protein per hr) Before Injection	After Injection
Saline	0.04	0.01
Deactivated ICSH	0.06	0.06
α-MSH	<0.01	<0.01
Growth hormone	0.03	<0.01
FSH	<0.01	<0.01
TSH	0.04	0 01
ACTH	0.08	0.06
Prolactin	0.02	<0.01

[a] Tyrosinase activity was measured before and 24 hr after administration of the substances shown in 0.1 ml of saline as described in Experimental Section. The hormones were administered in the dose of 100 μg except ACTH of which 5 units was used.

TABLE V: Tyrosinase Activity of Skin and Liver.[a]

Bird	Tyrosinase Activity (mμmoles hydroxylated/mg of protein per hr) Feather Tract	Skin	Liver
1	0.04	<0.01	0.4
2	<0.01	<0.01	0.8
3	0.1	<0.01	1.7
4	0.08	<0.01	1.2
5	<0.01	<0.01	1.4
6	<0.01	<0.01	0.9
7	2.1	<0.01	1.7
8	3.0	<0.01	1.1
9	1.8	<0.01	0.9
10	0.9	<0.01	0.3
11	2.1	<0.01	1.2
12	3.2	<0.01	1.6

[a] Seven days after plucking, birds 1–6 were given a single injection of saline intramuscularly and birds 7–12 were given ICSH 100 μg in saline. Twenty-four hours later the birds were sacrificed and tyrosinase activity was measured in the skin of feather tracts, the skin between feather tracts (column headed skin), and liver.

in the liver of these birds was not identified as tyrosinase by the criteria reported above for feather tracts.

Discussion

In conformation of previous observations (Okazaki and Hall, 1965), the present data indicate that the feather tracts of *S. paradisaea* contain small and variable amounts of tyrosinase. The nature of the enzymatic activity measured is indicated by the stoichiometry of the tritium exchange (dopa[^{3}H] *vs.* TOH) and by identification of the product of the reaction as dopa (Tables I–III). The radiochemical identity of the dopa formed (Tables II and III) provides strong evidence that the reaction measured involves the conversion of tyrosine to dopa.

The stimulation of enzyme activity by catalytic concentrations of dopa (Figures 1 and 2) is consistent with the behavior of tyrosinase from melanoma (Pomerantz, 1963, 1964) and from other sources (Lerner, 1953). The pteridine compound tested failed to stimulate the feather tract enzyme. These observations serve to

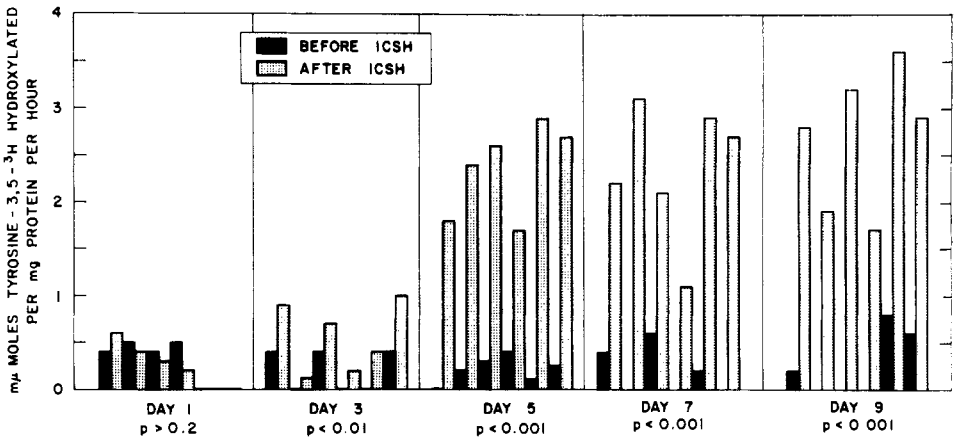

FIGURE 5: The effect of ICSH upon tyrosinase activity in feather tracts of *S. paradisaea*. Each pair of bars represents a single determination of enzyme activity on each of the two feather tracts of one bird—one before and one 24 hr after a single intramuscular injection of ICSH (100 μg). Each panel shows the results of experiments upon six birds performed on various days after plucking.

distinguish this enzyme from the tyrosine hydroxylase found in the adrenal medulla which is stimulated by pteridines (including 2,4-dihydroxy-5,6,7,8-tetrahydropteridine) (Ellenbogen *et al.*, 1965) but not by catalytic concentrations of dopa. It should be added, however, that only one pteridine compound was tested, and since the naturally occurring cofactor of tyrosinase has not been identified, some reservation concerning the specificity of the electron donors studied is necessary. Moreover, Pomerantz (1964) found that the pteridine used in these studies stimulated mammalian tyrosinase at high concentrations. Whether this difference reflects a species difference or resulted from the use of a crude enzyme system in the present experiments cannot be stated at present.

Some interest attaches to the influence of temperature upon avian tyrosinase since the body temperature of birds is significantly higher than that of mammals (Welty, 1962). Consequently, maximal activity might have been expected at perhaps 40° rather than 38° as observed here (Figure 3). However, the temperature of avian skin may be lower than that of deeper structures. The avian enzyme shows a broad peak of activity at pH 7.8 (Figure 4).

Within 24 hr of a single injection of ICSH, tyrosinase activity in feather tracts showed a considerable increase, provided that more than 1 day was allowed to elapse between plucking and injection (Figure 5). The increase in enzyme activity was more evident at longer times after plucking (Figure 5). These observations are in keeping with the action of ICSH upon gross changes in feather color. For example, Witschi (1940) reported that if ICSH was injected within the first 2 days after plucking, the newly erupted feathers did not show black bars; injection of ICSH 3 or more days after plucking produced a typical response. It was also found in the present experiments that tyrosinase activity was not increased during the first 12 hr after injection of ICSH (see Results). In studies to be reported elsewhere, it was found that the first detectable response to ICSH was observed microscopically in regenerating feathers 16 hr after injection of the hormone; this response took the form of a band of black melanocytes within the feather. Evidently both responses require similar conditions with respect both to time after plucking and time after injection.

The similarity between the two responses to ICSH (feather color and increased tyrosinase activity) is further demonstrated by the findings shown in Table IV. Of the hormones tested only ICSH increased tyrosinase activity. Segal (1957) reported that only ICSH and gonadotrophic hormones which show ICSH-like activity, produce black bars on regenerating feathers.

Failure of ACTH and α-MSH to stimulate tyrosinase activity (Table IV) is of interest in view of the fact that these hormones cause darkening of pelage of the weasel (Rust, 1965) and ACTH increases tyrosinase in goldfish (Chavin *et al.*, 1963). Before concluding that MSH is without effect in the present system, it may be necessary to use the avian hormone(s) since important differences in the MSH from pituitary extracts of various species has been reported (Burgers, 1963).

The specificity of the response to ICSH is further illustrated in Table V; neither liver nor breast skin from which feathers do not grow, showed an increase in tyrosinase activity after injection of the hormone.

Finally, the failure of ICSH to stimulate tyrosinase activity *in vitro* is in marked contrast to the intense stimulation of steroidogenesis which this hormone produces when added *in vitro* to corpus luteum (Mason *et al.*, 1962) and testis (Hall and Eik-Nes, 1962). The failure of ICSH to act *in vitro* upon tyrosinase activity is, however, in accord with the fact that a local action on feather color around the site of injection or implantation of ICSH could not be demonstrated (Hall *et al.*, 1965). These observations suggest that ICSH may not act

directly upon the feather tracts and, since the action of the hormone on feather color is extragonadal (Witschi, 1940, 1961), the site(s) of action of ICSH with respect to feather color and tyrosinase activity remain(s) to be determined.

Since ICSH stimulates tyrosinase activity in feather tracts under conditions similar to those in which the hormone causes blackening of feathers, it is likely that increased tyrosinase activity is, in part at least, responsible for the change in feather color caused by this hormone.

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