Increased Estrogen Formation and Aromatase Activity in Fibroblasts Cultured from the Skin of Chickens with the Henny Feathering Trait*

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The henny feathering trait in chickens leads to a marked increase in the conversion of androgen to estrogen in skin and other peripheral tissues with the result that feathers of affected males are feminized. To gain insight into the mechanisms responsible for this increased estrogen synthesis, we studied the conversion of testosterone to estrogen in fibroblasts cultured from the skin of control chickens and from two breeds carrying the henny feathering trait, the Sebright bantam and the Campine. Estrogen synthesis was measured in suspensions of intact fibroblasts and in cell-free fibroblast extracts by two assays: 1) direct measurement of 17β-estradiol formation from $[1,2,6,7-^3H]$testosterone, and 2) assessment of ^3H_2O release from $[1\beta-^3H]$testosterone. Both assays gave comparable results. Estrogen formation was as much as several hundredfold higher in fibroblasts cultured from skin of chickens carrying the henny feathering trait compared to that observed in fibroblasts from skin of control chickens. The current data indicate that increased estrogen formation in skin of chickens with the henny feathering trait is due to an enhanced activity of the aromatase complex of enzymes responsible for estrogen synthesis. The molecular basis for this increased activity is unclear.

Androgens can be converted to estrogens in many peripheral tissues including adipocytes (1, 2), hair follicles (3), and fibroblasts cultured from human skin (4). 17β-Estradiol is formed in these sites either directly from testosterone or indirectly via the sequence androstenedione → estrone → 17β-estradiol. Although this pathway accounts for only 1 % or less of androgen metabolism in the normal individual, it constitutes a major source of estrogen formation in target tissues such as brain (5, 6) and is the principal source of circulating estrogen in men and postmenopausal women (7). The factors that regulate the activity of this pathway are poorly understood.

We have recently demonstrated that the henny feathering trait in certain breeds of chickens is associated with a marked increase in the rate of conversion of androgen to estrogen in skin, skin appendages, and other peripheral tissues. As a result, the feathers of affected males are feminized and plasma estrogen levels are increased (8). Since this disorder may provide insight into the mechanisms by which estrogen formation is controlled in peripheral tissues, we examined estrogen synthesis in fibroblasts cultured from the skin of control

chickens and of two breeds carrying the henny feathering trait. The results demonstrate that estrogen formation is markedly increased in fibroblasts cultured from skin of chickens with the henny feathering trait and that this increase is due to an enhancement of the activity of the enzyme complex (estrogen synthetase or aromatase) responsible for estrogen synthesis.

MATERIALS AND METHODS[1]

[1] Portions of this paper (including "Materials and Methods," Figs. 1-7, and Table I) are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20014. Request Document No. 80M-2434, cite authors, and include a check or money order for $6.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

was empirically adopted as a standard technique for growing fibroblasts from chicken skin:

3 mm punch biopsies of either breast or back skin from the various breeds of chickens were cut into 1-2 mm fragments; the fragments were placed in 25 cm² primary culture flasks containing 5 ml of Dulbecco's modified Eagle's medium:medium 199, 4:1 (v/v), pH 7.4, supplemented with 0.8 mM sodium pyruvate, 40 mM sodium bicarbonate, 100 µU/ml penicillin, 100 µg/ml streptomycin (DME-199), 7.5 percent chicken serum, and 7.5 percent fetal calf serum (FCS). Flasks were incubated in a humidified atmosphere containing 5 percent CO_2 at a temperature of 41°C. After 8 to 10 days in culture, the primary fibroblast monolayers were rinsed with 5 ml of phosphate buffered saline (PBS), dissociated by exposure to 1 ml of 0.05 percent trypsin - 0.03 percent EDTA at 37°C for 3 min, and transferred to 75 cm² stock culture flasks containing 10 ml of DME-199 supplemented with 5 percent chicken serum and 5 percent FCS. The flasks were returned to a 41°C incubator, and the fibroblasts were allowed to grow approximately 6 to 8 days until confluent monolayers were obtained. Under these conditions, the doubling time of fibroblasts is 18-20 h. The cells were then rinsed twice with 3 ml of PBS and dissociated by exposure to 2 ml of 0.05 percent trypsin - 0.03 percent EDTA at 37°C for 3 min and either (a) divided and returned to stock flasks or (b) plated (day 0) at a density of 2-3 x 10⁵ cells/dish in 10 cm diameter culture dishes for subsequent use in enzyme assays. All cells were grown at 41°C and utilized before transfer number 35, as estimated from the average doubling time.

In some experiments original stock cultures were used, and in others the fibroblasts were frozen and subsequently thawed and propagated for study. For the latter purpose, confluent stock culture flasks (transfer number less than 20) were rinsed twice with 5 ml PBS. 4 ml of 0.025 percent trypsin - 0.015 percent EDTA were then added, and the flasks were incubated at 37°C for 3 min. The cells from each flask were collected in separate tubes, pelleted by centrifugation at 800g for 3 min, and resuspended in 2 ml of DME-199 containing 5 percent chicken serum, 5 percent FCS, and 5 percent dimethyl sulfoxide. 1 ml aliquots of the cell suspension were transferred to 2 ml glass vials. The vials were placed in a freezer (-20°C) for 30-45 min and then transferred to liquid nitrogen. To replenish stock cultures, vials were removed from liquid nitrogen and quickly thawed in a 37°C water bath, the contents of each vial were transferred to a 75 cm² flask with 10 ml of DME-199 containing 5 percent chicken serum and 5 percent FCS, and the flasks were placed in the 41°C incubator. Medium and serum in each flask were replaced with 10 ml of fresh medium and serum after 24 h.

To grow cells for enzyme assay fibroblasts were plated in culture dishes containing 10 ml DME-199, 5 percent chicken serum and 5 percent FCS; fresh medium and serum were added on days 3 and 6 of culture. On day 7 the confluent fibroblast monolayers were harvested by trypsinization (for whole cell assays) or by scraping (for subcellular particulate fraction assays).

Assay of Aromatase in Intact Fibroblasts

Fibroblast monolayers were rinsed twice with 3 ml of 50 mM Tris-Cl, pH 7.4, in 150 mM NaCl (Tris-saline). Cells were dissociated by incubation with 1.5 ml 0.025 percent trypsin - 0.015 percent EDTA at 37°C for 3 min. Following neutralization of the trypsin with 1.5 ml lima bean trypsin inhibitor (0.5 mg/ml), the cells were collected and centrifuged at 800g for 10 min at 4°C. The supernatant was discarded, and the pellet was resuspended in MEM to give a final protein concentration of 1-1.3 mg/ml.

0.25 ml of the cell suspension was added to 13x100 mm screw-top tubes containing [1,2,6,7-³H]testosterone in 0.25 ml MEM so that the final testosterone concentration varied from 0.005 to 0.5 µM. The tubes were stirred, gassed with 95 percent O_2/5 percent CO_2 for 15 s, and incubated with shaking in a slanted rack at 37°C for 30 min. The reaction was terminated by transferring the tubes to an ice bath. After addition of 17β-[4-¹⁴C]estradiol and [4-¹⁴C]estrone (approximately 4000 dpm each) the steroids were extracted from the reaction mixture with 5 volumes of ethyl acetate, and solvolysis was performed by a modification (8) of the method of Burstein and Lieberman (11). The solvolyzed extracts were dried under nitrogen and redissolved in 5.0 ml 2 percent ethyl acetate in isooctane prior to celite chromatography. Estrone and 17β-estradiol were separated on small celite columns and purified to homogeneity by successive thin layer chromatography of the acetate derivatives as described previously (8,12).

The protein in the aqueous phase was precipitated at 4°C with 5 volumes of 10 percent trichloroacetic acid and centrifuged at 800g for 20 min. The pellet was dissolved in 0.05N NaOH, and aliquots were taken for protein measurement by the Lowry method (13).

Assays of Aromatase in Particulate Fractions of Cells

Fibroblast monolayers were rinsed twice with 3 ml of Tris-saline. Cells were scraped into 2 ml of Tris-saline and centrifuged at 800g for 10 min. The pellet was rinsed twice with Tris-saline and resuspended in 10 mM Tris-Cl, pH 7.4. The cells were disrupted by three 5-s periods of sonication with a Biosonic III probe sonicator at a power of approximately 50 watts. The resulting homogenate was centrifuged at 800g for 10 min, and the supernatant was aspirated and centrifuged at 105,000g for 60 min. The supernatant was discarded, and the pellet was resuspended in 10 mM Tris-Cl, pH 7.4, with a Dounce homogenizer. Aliquots of this particulate suspension were used for enzyme assay and for protein determination by the Lowry method (13).

Two different assays of aromatase activity were utilized in the particulate suspension. In the initial experiments, aliquots (0.1 to 0.4 mg protein) were incubated with 0.1 µM [1,2,6,7-³H]testosterone, 0.18 µM 17β-[4-¹⁴C]estradiol (approximately 4000 dpm), 5 mM NADPH, 0.1 M Tris-Cl - 0.1 M sodium citrate, pH 6.5, in a total volume of 0.2 ml. Following incubation for 60 min at 25°C an abbreviated procedure was utilized for the isolation of radioactive 17β-estradiol. In brief, the procedure involves extraction of the incubation mixture and the formation and subsequent purification of 17β-estradiol diacetate by successive thin layer chromatography (12).

The standard assay of aromatase activity in particles measured the release of ³H₂O from [18-³H]testosterone during estrogen synthesis by a modification of the procedure of Thompson and Siiteri (14). The assay mixture contained 0.25 µM [18-³H]testosterone, 5 mM NADPH, 0.1 M Tris-Cl - 0.1 M sodium citrate buffer, pH 6.5, and 0.1 to 0.4 mg protein in a final volume of 0.2 ml. Incubations were performed at 25°C for 60 min. Reactions were terminated by addition of 5 volumes of chloroform. 1.3 ml of 10 percent trichloroacetic acid were added, and the tubes were centrifuged at 800g for 5 min. 1.0 ml aliquots of the aqueous supernatant were removed and added to 1.0 ml of 5 percent dextran-coated charcoal; the samples were mixed and centrifuged at 800g for 10 min. 1.0 ml of the supernatant was pipetted into scintillation vials containing 10 ml of Picofluor 15, and ³H was assayed in a Packard 2650 scintillation counter.

For determination of pH optimum, the pH was varied from 4.5 to 9.5. The apparent K_m for testosterone was measured by varying the concentration of testosterone from 0.01 to 1.0 µM. The apparent K_m for NADPH was assessed at concentrations of NADPH that varied from 0.001 to 5.0 mM in the presence of 0.25 µM testosterone. Both apparent K_m values were calculated from double reciprocal plots using the method of least squares.

Other Enzyme Assays

To measure the rate of 5α-reduction of testosterone during the incubations of intact fibroblasts and subcellular particles with [1,2,6,7-³H]testosterone, 50 µl aliquots of the ethyl acetate extracts were evaporated to dryness after addition of 10-20 µg each of nonradioactive androstanedione, Δ⁴-androstenedione, dihydrotestosterone, testosterone, and 3α-androstanediol. This residue was dissolved in 20 µl of chloroform, spotted on plates, and separated by thin layer chromatography as described previously (15). The percentage of the total radioactivity in 5α-reduced steroids (androstanedione, dihydrotestosterone, and 3α-androstanediol) was used to determine 5α-reductase activity (15).

NADPH-cytochrome c reductase was measured by a modification of the method of Masters, Williams and Kamin (16). Subcellular particles from fibroblast homogenates were prepared as described above and resuspended in 0.1 M KP_i - 0.1 mM EDTA (pH 7.7) with a Dounce homogenizer. The assay mixture contained 0.033 mM cytochrome c, 0.83 mM KCN, 0.5 or 1 mM NADPH, and particles (0.04-0.28 mg protein) in a total volume of 1.2 ml KP_i-EDTA buffer pH 7.7. NADPH-cytochrome c reductase activity was calculated as a function of the change in absorbance at 550 nm at room temperature.

RESULTS

The conversion of [1,2,6,7-³H]testosterone to radioactive estrogen was first assessed in suspensions of intact fibroblasts grown from the skin of Sebright and Leghorn bantam chickens (Fig. 1). (Since more than 98 % of the ³H in the 1 and 2 positions of [1,2,6,7-³H]testosterone is in the α position and is not lost during aromatization (3), this substrate is suitable for assessing estrogen formation from testosterone.) Maximal rates of estrogen formation in skin fibroblasts from Sebright chickens occurred between 0.1 and 0.25 µM testosterone, similar to the saturating concentration of substrate for estrogen synthesis in chicken skin (8). Ninety-seven percent of the estrogen formed in the Sebright cells was 17β-estradiol (1600 fmol/h/mg of protein), and the remainder was recovered as estrone (50 fmol/h/mg of protein). In one experiment the radioactive 17β-estradiol diacetate recovered from the final thin layer preparation was mixed with 40 mg of 17β-estradiol diacetate, and the mixture was recrystallized 5 times from ether/petroleum ether; there was less than a 6 % change in ³H to ¹⁴C ratio during the recrystallization. In another experiment the radioactive estradiol diacetate purified by thin layer chromatography was hydrolyzed and separated into 17β-estradiol and 17α-estradiol as before (8); more than 99 % of the ³H was recovered in the area corresponding to 17β-estradiol. We therefore concluded that this procedure is suitable for purification of 17β-estradiol from fibroblasts. Furthermore, in contrast to intact chicken skin (8), fibroblasts cultured from chicken skin contain little 17β-hydroxysteroid dehydrogenase (as reflected by low rates of conversion of 17β-estradiol to estrone). Therefore, measurement of the conversion of testosterone to 17β-estradiol provides a valid assessment of estrogen formation in Sebright fibroblasts. In contrast to the high rates in Sebright fibroblasts, low rates of synthesis of both 17β-estradiol and estrone were observed in fibroblasts derived from skin of the Leghorn bantam (Fig. 1). Thus, the profound difference in rates of estrogen formation between the skin of Sebright and of control chickens (8) is also expressed in skin fibroblasts.

Aromatase was then assayed in suspensions of subcellular particles prepared from sonicates of control and Sebright
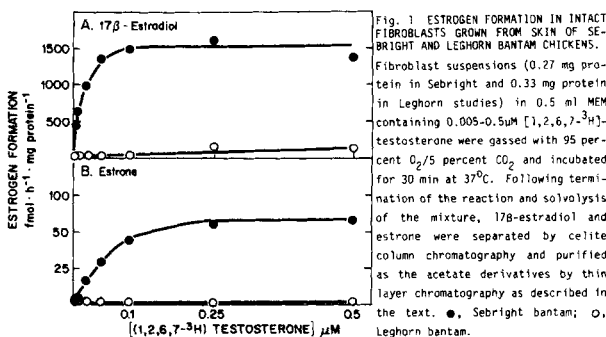
Fig. 1 ESTROGEN FORMATION IN INTACT FIBROBLASTS GROWN FROM SKIN OF SEBRIGHT AND LEGHORN BANTAM CHICKENS. Fibroblast suspensions (0.27 mg protein in Sebright and 0.33 mg protein in Leghorn studies) in 0.5 ml MEM containing 0.005-0.5µM [1,2,6,7-³H]-testosterone were gassed with 95 percent O_2/5 percent CO_2 and incubated for 30 min at 37°C. Following termination of the reaction and solvolysis of the mixture, 17β-estradiol and estrone were separated by celite column chromatography and purified as the acetate derivatives by thin layer chromatography as described in the text. ●, Sebright bantam; ○, Leghorn bantam.

Fig. 2 COMPARISON OF TWO ASSAYS OF ARO-MATASE ACTIVITY IN FIBROBLAST PARTICLES.
A. ^3H_2O RELEASE FROM [18-^{3}H]TESTOSTERONE. Fibroblast particles (0.41 mg protein in Sebright and 0.27 mg protein in Leghorn studies) were incubated in 0.2 ml Tris-citrate, pH 6.5, containing 5 mM NADPH and 0.01-1μM [18-^{3}H]testosterone for 60 min at 25°C. ^3H_2O was assayed as described in the text. B. 17β-ESTRADIOL FORMATION FROM [1,2,6,7-^{3}H]TESTOSTERONE. Fibroblast particles (0.41 mg protein in Sebright and 0.27 mg protein in Leghorn) were incubated in 0.2 ml Tris-citrate, pH 6.5, containing 0.13 μM 17β-[4-^{14}C]estradiol, 5 mM NADPH and 0.01-1μM [1,2,6,7-^{3}H]testosterone for 60 min at 25°C. 17β-Estradiol was extracted, converted to the diacetate, and purified as described in the text. ●, Sebright bantam; O, Leghorn bantam.

fibroblasts (Fig. 2). As assessed by the release of the 1β equatorial hydrogen from [1β-^{3}H]testosterone into water during aromatization (14), estrogen formation was again maximal in the Sebright preparation at a testosterone concentration of 0.25 μM; aromatase activity in the control preparation was low (Fig. 2A). In parallel experiments the rates and saturation kinetics of 17β-estradiol formation from [1,2,6,7-^{3}H]testosterone in the fibroblast particles (Fig. 2B) were similar to those observed with the water assay. We concluded that the ^3H_2O assay could be used in this system to assess estrogen formation. The apparent K_m of the reaction in subcellular particulate fractions of Sebright fibroblasts averaged 0.021 μM (with a range of 0.011 to 0.036 μM) in 3 determinations. For unclear reasons, however, the V_{max} of the reaction in preparations of particles from different Sebright fibroblast cultures (even those derived from the same animal) varied by as much as 30- to 40-fold.

Aromatase activity is more linear at 25 °C than at 37 °C (Fig. 3A). At 25 °C the reaction is linear for at least 60 min and with amounts of protein up to 0.3 mg/assay (Fig. 3B). The optimal pH of the aromatase reaction is between pH 6.5 and 7.0 (Fig. 4). The saturating concentration of NADPH is approximately 0.05 mM with an apparent K_m of 0.02 mM (Fig. 5).

The intracellular distribution of aromatase was determined by assaying activity in th whole cell homogenate and in various fractions obtained after differential centrifugation (Table I). Approximately 85% of the activity was recovered in the particulate fraction; as in placenta, approximately half the activity was in the 10,000 × g pellet, and the remainder was in the 105,000 × g pellet (17–19). Therefore, the particulate fraction obtained by centrifuging the 800 × g supernatant at 105,000 × g was used for the standard assay.

We then assessed aromatase in fibroblast particles obtained from a nonbantam breed of chicken that carries the henny feathering trait (the golden Campine) and from a nonbantam control strain (the white Leghorn) (Fig. 6). Major differences were again observed between henny-feathered and control

birds as assessed both by the ^3H_2O assay and by the direct measurement of 17β-estradiol formation. Since the rates of aromatization in particles from both the full size and bantam Leghorn were equally low, we concluded that the bantam trait does not influence aromatase activity in skin fibroblasts.

These studies demonstrate that increased estrogen formation in the animals with the henny feathering trait is due to an increase in the aromatase enzyme itself. The difference in the amount of this enzyme between cells cultured from normal and henny-feathered birds is apparently not due to degradation of enzyme in normal cells, since mixtures of subcellular particles from control and Sebright demonstrate intermediate levels of enzyme activity (results not shown). Aromatase ac-

Fig. 3. AROMATASE ACTIVITY IN FIBROBLAST PARTICLES AS A FUNCTION OF TEMPERATURE, TIME, AND PROTEIN.
A. Fibroblast particles (0.17 mg protein in Sebright and 0.25 mg protein in Leghorn studies) were incubated in 0.2 ml Tris-citrate, pH 6.5, containing 5mM NADPH and 0.25 μM [18-^{3}H]testosterone for 60 min at either 25°C or 37°C. ■, Sebright bantam, 37°C; ●, Sebright bantam, 25°C; O, Leghorn bantam, 25°C and 37°C. B. Fibroblast particles (0.043-0.33 mg protein in Sebright and 0.066-0.49 mg protein in Leghorn studies) were incubated in 0.2 ml Tris-citrate, pH 6.5, containing 5 mM NADPH and 0.25 μM [18-^{3}H]testosterone for 60 min at 25°C. ^3H_2O was assayed as described in the text. ●, Sebright bantam; O, Leghorn bantam.

Fig. 4 AROMATASE ACTIVITY IN FIBROBLAST PARTICLES AS A FUNCTION OF pH.
Particles (0.17 mg in Sebright and 0.25 mg in Leghorn studies) were incubated in 0.2 ml Tris-citrate (pH range 4.5-9.5) containing 5 mM NADPH and 0.25 μM [18-^{3}H]-testosterone for 60 min at 25°C. ^3H_2O was assayed as described in the text. ●, Sebright bantam; O, Leghorn bantam.
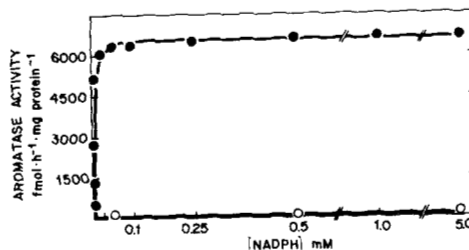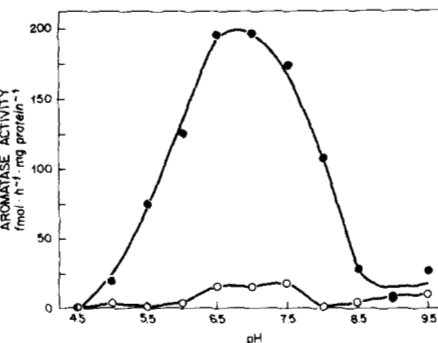
Fig. 5 AROMATASE ACTIVITY AS A FUNCTION OF NADPH CONCENTRATION.
Fibroblast particles (0.15 mg protein in Sebright and 0.14 mg protein in Leghorn studies) were incubated for 60 min at 25°C in 0.2 ml Tris-citrate buffer, pH 6.5, containing 0.25 μM [18-^{3}H]testosterone and 0.001-5 mM NADPH. ^3H_2O was assayed as described in the text. ●, Sebright bantam; O, Leghorn bantam.

Table I. INTRACELLULAR DISTRIBUTION OF AROMATASE IN FIBROBLASTS

SUBCELLULAR FRACTION	AROMATASE ACTIVITY		
	SEBRIGHT FIBROBLASTS		LEGHORN FIBROBLASTS
	fmol·h⁻¹	fmol·h⁻¹·mg protein⁻¹	fmol·h⁻¹
Cell homogenate	350	2500	<10
800g pellet	<10	<100	<10
10,000g pellet	150	4500	<10
105,000g pellet	140	3100	<10
105,000g supernatant	20	250	<10

Various cell fractions (containing 0.01-0.14 mg protein in Sebright and 0.01-0.09 mg protein in Leghorn bantam studies) were incubated with 0.25 μM [18-^{3}H]testosterone, and the release of ^3H_2O was measured as described in the text for the standard assay.
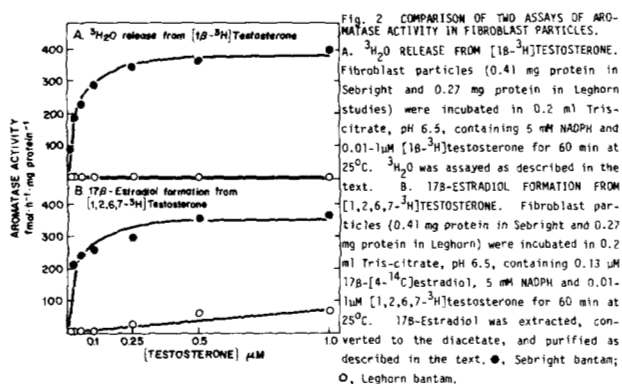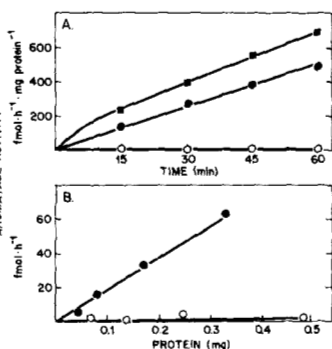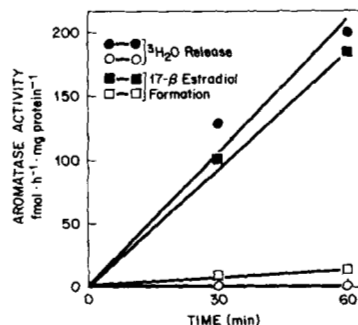

Fig. 6 AROMATASE ACTIVITY IN FIBROBLASTS GROWN FROM SKIN OF CAMPINE AND LEGHORN CHICKENS.
Fibroblast particles (0.17 mg protein in Leghorn and 0.39 mg protein in Campine studies) were incubated at 25°C for 30 or 60 min in 0.2 ml Tris-citrate buffer, pH 6.5, containing 5 mM NADPH and either 0.1 μM [1,2,6,7-^{3}H]testosterone or 0.1 μM [18-^{3}H]testosterone. At the end of the incubation either 17β-[^{3}H]estradiol diacetate was formed and purified, or ^3H_2O was assayed as described in the text. ● and ■, Campine; O and □, Leghorn.

Estrogen Formation in Chicken Fibroblasts

Fig. 7 AROMATASE ACTIVITY AND PROTEIN CONTENT AS A FUNCTION OF CELL GROWTH. Sebright and Leghorn bantam fibroblasts were plated in 10 cm Falcon dishes at a density of 150,000 cells per dish and propagated at 41°C as described in the text. Cells were harvested, and arom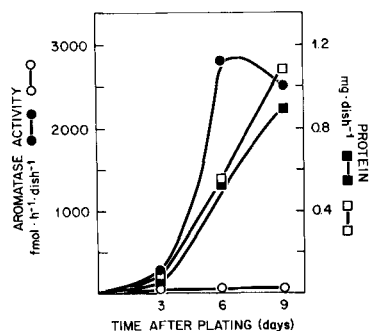atase activity in the particles was assayed using the ^3H_2O assay on days 3, 6, and 9. Protein concentration was measured in the whole homogenates from which the subcellular particles were prepared. ● and ■, Sebright bantam; ○ and □, Leghorn bantam.

tivity in Sebright cells increases for 6 to 8 days after plating, and as cells become confluent, total enzyme activity plateaus (Fig. 7). The difference in aromatase activity between control and Sebright cells is not characteristic of all fibroblast enzymes, since there was no difference in steroid 5α-reductase or NADPH-cytochrome c reductase between the two types of cells (results not shown).

DISCUSSION

The henny feathering trait in the chicken is associated with a striking increase in the rate of estrogen synthesis in skin (8) and in fibroblasts cultured from skin. This trait is expressed in peripheral tissues throughout the life of the chicken (8) and is hereditable, but whether it is due to one or two mutant genes is uncertain (20, 21). Our findings suggest that the henny feathering is due to increased activity of the complex of enzymes involved in the aromatization of androgens, although the exact component or components responsible for the increased activity has not been identified. These observations are of physiologic interest since the factors that regulate the rate of aromatase activity in peripheral tissues are not understood.

Within a given tissue such as brain, the rate of estrogen formation varies enormously at different stages of development (6), and the overall rates of the reaction in the body also vary as a function of age and body weight (7). Furthermore, in rare feminizing disorders in the human male, peripheral aromatase activity can be increased more than 40-fold (22). Identification of a genetic trait that results in a striking increase in the rate of peripheral aromatization raises the possibility that allelic variations at one or more gene loci control this process under normal circumstances.

Several mutations result in increased enzyme activity. A rare variant of glucose-6-phosphate dehydrogenase (G6PD Hektoen) is due to a mutation that alters the structure of the enzyme and simultaneously increases the catalytic activity of each enzyme molecule (23, 24). Likewise, a mutation of the human phosphoribosyl-pyrophosphate synthetase results in increased activity per molecule (25). On the other hand, the increase in β-hydroxy-β-methylglutaryl coenzyme A reductase in familial hypercholesterolemia is the consequence of an increase in the synthetic rate of the enzyme (26). In the case of other mutant enzymes with enhanced activity such as

adenosine deaminase (27), it is not established whether the increased activity is due to an increased amount of normal protein or the presence of a mutant protein with enhanced catalytic activity per molecule. This uncertainty also extends to the aromatase enzyme in the Sebright and Campine fibroblasts. Enzymatic activity is so low in the normal cells that it is not possible to be certain whether the kinetic properties are identical in normal and mutant cell lines. Elucidation of the molecular mechanism of this abnormality may provide insight into the normal control mechanisms that regulate estrogen formation in peripheral tissues.

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