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The Serum Ferroxidase Activity and the Iron Mobilization by Estrogens*

J. Planas

Laboratory of Animal Physiology
Faculty of Science
University of Barcelona
Barcelona - 7 (Spain)

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Serum iron and serum ferroxidase activity has been studied in hens and roosters. It has been observed in sexual mature females, there are a parallel increase of both parameters, that reachs a maximum in the laying period with a sevenfold and a twenty-fold increase respectively.

Serum iron and copper rise also in a parallel way during the laying period and a similar increase was observed in immature pullets after estradiol injection.

The administration of serum gonadotrophin in immature pullets produce a rise in the ferroxidase activity, similar to that obtained by diethylstilbestrol injection.

The ferroxidase induction by estrogens exogenous or by an endogenous estrogens (laying period or gonadotrophin injections) seems the same phenomenum.

From the data brought out by FRIEDEN and his group (19) it has been proposed that the ceruloplasmin, a plasma copper protein, provides a ferroxidase activity that catalizes the oxidation of Fe(II) to Fe(III) which facilitates the formation of the transferrin-Fe(III) complex.

The experimental evidence *in vivo* supported by the University of UTAH group (27, 31) and the experiments in perfused liver by the same FRIEDEN's group (20) have shown without doubt that the ceruloplasmin (Ferroxidase I) is essential for the mobilization of iron from cells to plasma and this protein acts through its ferroxidase activity.

The role of the ceruloplasmin in the normal iron metabolism is now clear and this cupro-enzyme represents the molecular link between copper and iron metabolism (8).

It is known that the estrogens exert a stimulating effect on copper and ceruloplasmin concentrations in rats (7, 18, 34) and in normal persons (32, 33) or in

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women taking estrogens as oral contraceptives (5, 6).

On the contrary, the estrogenic effect on plasma iron in mammals is not universal and the sexual differences in some species are difficult to explain from an endocrinological point of view. The administration of estrogens produces a decrease of the plasma iron in rabbits (15, 17, 21). In rats, the females have higher values than males and the ovariectomy reduces such difference (13), as it was observed in horses as well (26). In humans, estrogen administration produces different effects in men and women and the results found in the bibliography are not conclusive (17). However, it seems evident that its use as oral contraceptive produces a significant elevation of the plasma iron in women (3, 16).

In birds, the estrogens increase the plasma iron (4, 10, 29) and this effect is general because it is associated with egg formation (22, 28).

The laying period represents a time of tremendous increase in the iron metabolism reflected in a twofold rise of plasma iron turnover (2, 23) since the bird at that time has a double need for iron: for the hemoglobin synthesis and for the egg formation (11).

The ferroxidase system in male chickens has been studied in both normal ones, with copper and iron deficiency as well as in estrogenized roosters (25).

The present work brings more data about the idea to analyze the physiological effect of the estrogens on the ferroxidase system in fowl.

Materials and Methods

The serum iron has been determined by method of Ramsay (30).

The serum ferroxidase activity has been evaluated according to JOHNSON *et al.* (12), as in a previous paper (25), but using an Hitachi-Perkin Elmer, Mod. 139, adapted to a Perkin-Elmer Recorder, Mod. 56. The reaction mixture was 1.475 ml [0.050 ml fresh serum; 0.6 ml acetate buffer 0.364 M,

Table I. *Serum iron and copper values in chickens and turkeys in groups of different sex with special reference in the laying state.*
Mean values ± S. D.

Species	No.	Serum iron µg Fe/100 ml	Serum copper µg Cu/100 ml	Correlations Fe — Cu
CHICKENS				
Males	20	117 ± 40	21 ± 5	$r = 0.72$ $p < 0.01$
Females adult not laying	7	170 ± 38	30 ± 8	$r = 0.91$ $p < 0.001$
Females laying	12	466 ± 75	85 ± 25	$r = 0.64$ $p < 0.01$
TURKEYS				
Males	13	156 ± 39	37 ± 20	$r = 0.67$ $p < 0.001$
Females adult not laying	5	127 ± 17	33 ± 9	$r = 0.88$ $p < 0.001$
Females laying	4	701 ± 118	77 ± 10	$r = 0.84$ $p < 0.01$

*p*H 6; 0.375 ml apotransferrin 1 % (Berringer); 0.45 ml Fe(II) 400μM].

In some samples too, the serum copper has been evaluated by a spectroscopic technique with a Boehringer kit (Mannheim, Germany).

White Leghorn chickens were used in this work, except the groups of adult hens (9 month old) laying and not laying, that were Arbor Acres Red. The turkeys were a normal black strain coming from Bañolas (Gerona). In the laboratory all the animals received a comercial diet and water *ad libitum*.

The hormones used were: a) Diethylstilbestrol (Merck) dissolved in 1,2-propanediol, in doses from 2 to 10 mg/kg b.w.; b) Estradiol benzoate (Progynon B, Schering) dose 3 mg/kg; c) Mare serum gonadotrophine (Antex Leo) in dose 30 and 60 U.I./animal.

Results

The correlation between serum iron and copper with a simultaneous increase during the laying period is evident (Table I).

The parallel increase of serum iron and copper in the laying period is a consequence of the higher estrogen level in this period. An evidence of this assertion can be obtained by injection of estradiol benzoate (3 mg estradiol, three times a week during two weeks) in a lot of 6 immature pullets (Fig. 1) that produce a sixfold increase of serum and copper, which remain constant during the second week and return to the base line values after a third week without treatment.

The serum ferroxidase activity in females is increased with sexual maturity. During the laying period a maximum is reached representing a twentyfold increa-

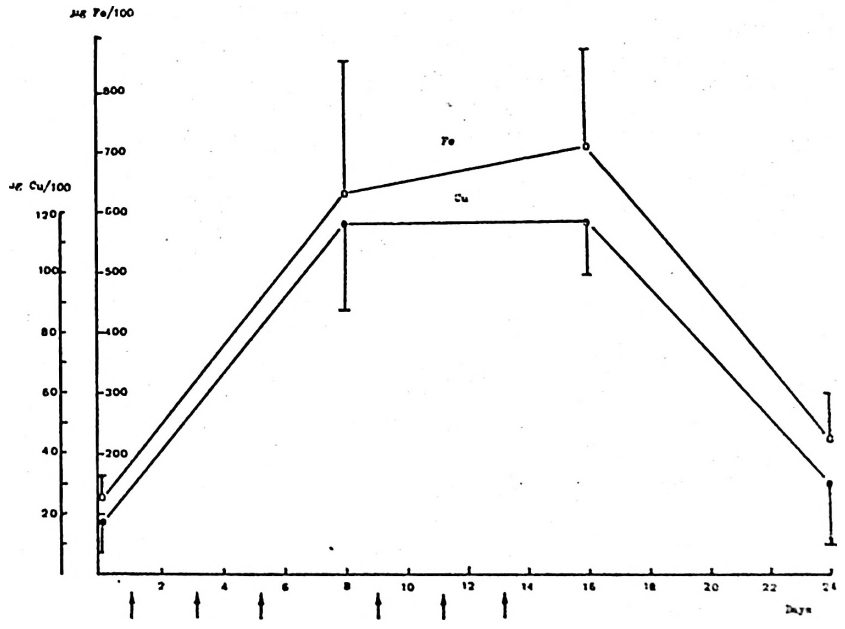

Fig. 1. *Effect of estradiol benzoate on serum iron and copper in a group 6 immature pullets.*

Schedule injection: one intramuscular injection (3 mg estradiol benzoate) three times a week during two weeks; see the arrows in the figure. Each point is the average of 6 specimens and the standard deviation is also shown.

Table II. *Serum Iron and ferroxidase activity in different groups of chickens. The azide (N_3^-) inhibition was obtained with a final concentration of 5 mM, added 3-5 minutes before the ferroxidase assay was begun.*
Mean values ± S.D.

Group	No.	Serum Iron μg Fe/100 ml	Ferroxidase activity	
			μM Fe(II)/min/ml	N_3^- Inhibition %
I. — Males and females (1-2 months old)	33	116 ± 27	31 ± 9	21
II. — Females (4 months old)	8	145 ± 31	76 ± 41	59
III. — Females laying (9 months old)	12	741 ± 139	633 ± 134	83
IV. — Females not laying temporary (9 months old)	6	655 ± 141	42 ± 20	81
V. — Males (4 months old)	10	180 ± 53	36 ± 12	15

Significance of differences:
Serum Iron: I-V, $p < 0.001$; I-II, $p < 0.05$; II-III, $p < 0.001$; II-IV, $p < 0.001$; III-IV, not significant; II-V, not significant.
Ferroxidase: I-II, $p < 0.001$; I-V, not significant; II-III, $p < 0.001$; II-IV, not significant; III-IV, $p < 0.001$; II-V, $p < 0.02$.

se; at the same time, the plasma iron increases to sevenfold the base line values (Table II).

An interesting observation was made in a group of hens that had just stopped laying for 2 or 3 days (Table II); the ferroxidase activity fell abruptly while the plasma iron remained high.

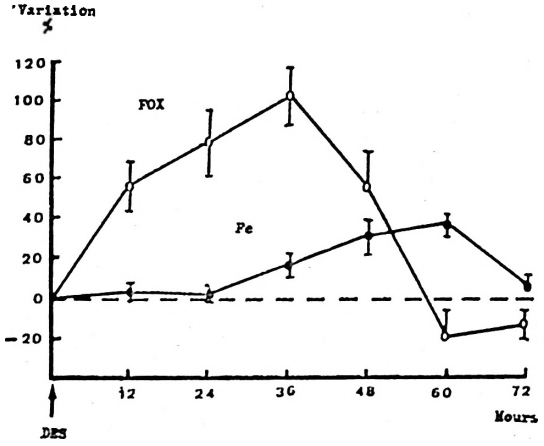

Fig. 2. *Serum ferroxidase activity (Fox) and the serum iron (Fe) variations from the base line values produced by a single injection of diethylstilbestrol (2 mg DES/kg).*
Each point is the average of 6 specimens with the standard deviation.

The estrogen administration to a group of 6 immature pullets has demonstrated that there is at first an increase in the ferroxidase activity arriving at a maximum at the 36th hours, and a peak in the plasma iron at the 60th hours (Fig. 2).

The induction effect of the exogenous on the ferroxidase system can be reproduced in experiments by administering gonadotrophin to immature pullets (Fig. 3). The results obtained provide an evidence to be considered as identical the effect produced by exogenous or endogenous estrogens.

Discussion

The laying period produces a double increase in iron and copper serum and this observation can be reproduced experimentally in the laboratory by estrogen admi-

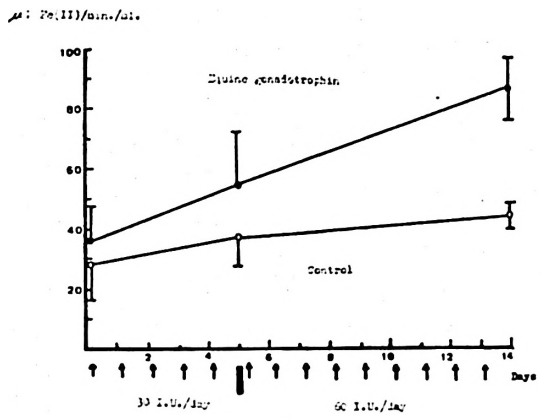

Fig. 3. *Effect on the serum ferroxidase activity of a daily injection of serum mare gonadotrophin in a lot of 6 inmature pullets (2 months old; 1.2-1.5 kg) against a control group with the same number of specimens and conditions but receiving only the solvent (saline).*

Mean values and standard deviations. Differences: on the 5th day, no significant; on the 14th day, $p < 0.001$.

nistration. An advance of these results has been already published (1). A significant correlation between serum iron and copper has been also found in several mammals and birds (24) and this correlation in fowl is not only found in serum but in eggs too (14).

In the females the serum ferroxidase activity rises with sexual maturity and reaches its highest level during the laying period. This results was expected after the previous observations in estrogenized roosters (25) where was demostrated that the hormone at first increase the ferroxidase activity on the second day after the injection and the plasma iron peak was reached on the third day, while the transferrin remains constant. The fast response of the ferroxidase system has now been appreciated in a shorter blood sampling schedule and the same pattern has been shown in the variation of both parameters (serum iron and ferroxidase).

As a work hypothesis it has been suggested (25) that the estrogen induces the synthesis of ceruloplasmin as a primary effect and the iron mobilization as its consequence. Then, if a laying hen stopped egg production on account of the interuption of estrogen secretion, as show in Table II, the immediate effect is the cessation of ferroxidase synthesis.

The identity of effect between 17β-estradiol and diethylstilbestrol has been also established earlier (25). The serum ferroxidase rises by the induction effect of exogenous estrogen on the ferroxidase system and it can be reproduced in experiments by administering gonadotrophin to immature pullets. The results obtained provide evidence to consider as identical the effect produced by exogenous or endogenous estrogens. Now there are further arguments to support an induction of ferroxidase by estrogens.

The ferroxidase system in chickens can be formed for two or more ferroxidases as it is the case in human beings (35) and which can be suspected by the different response to the azide inhibition. So the laying hens have a more sensitive ferroxidase system to azide reminding human ceruloplasmin (Ferroxidase I).

The bird is considered as an animal with the highest iron turnover (2, 23) according to the double need of iron. An efficient ferroxidase system is increased on estrogen induction and by means of this increase the iron is supplied to the reticulocytes for hemoglobin synthesis and by oviduct for egg formation. On the other hand, the plasma iron transport has been developed in a different line from that of the mammals, since the estrogens do not increases the transferrin (25) but induce the liver synthesis of a plasma phosphoprotein, phosvitin (10), an auxiliary iron-binding mechanism in non-mammal vertebrates with a decisive role in birds (22).

The funcional relationship between ceruloplasmin, transferrin and hemoglobin established by FRIEDEN (8, 9) was maintained in the birds with the special cha-

racteristic of the transport system and the reproductive regime.

On the other hand, it is interesting to point out that the ceruloplasmin induction by estrogens seems an universal phenomenum but the differences in iron mobilization in mammals by sex hormones may find an explanation with a more complete study of the ferroxidase system of various species.

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Resumen

Se ha valorado el contenido en hierro sérico y la actividad ferroxidasa (ceruloplasmina) en sueros de gallinas y pollos en distintos estados sexuales. Se observa como existe un crecimiento paralelo de estos parámetros que pueden relacionarse con la madurez sexual. Durante el período de puesta se alcanza un punto máximo con incrementos que representan unas siete veces y veinte veces, respectivamente.

Igualmente se observa en el período de puesta como el incremento del hierro sérico es paralelo al del cobre. Una idéntica respuesta se aprecia con la inyección de benzoato de estradiol en gallinas jóvenes.

La administración repetida de gonadotrofina sérica en gallinas inmaturas produce una elevación significativa de la ferroxidasa sérica.

El incremento de la actividad ferroxidasa del suero producida por estrógenos exógenos o endógenos (período puesta o inyección de gonadotrofina) parece un mismo fenómeno.

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