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The Effect of Estrogens on Serum Ferritin Levels in Duck

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Serum and tissue ferritin content is measured in duck by a RIA method before and after treatment with estrogens, as well as serum ferritin in laying and non-laying hen. Both serum ferritin and tissue ferritin decrease after treatment with estrogens, while serum iron increases. A relationship between serum ferritin and iron stores in duck is shown.

Key words: Duck, Serum ferritin, Tissue ferritin.

Serum ferritin concentration in humans is a good indicator of iron stores in extreme situations of iron status, as hemochromatosis or severe iron deficiency anemia (8). A correlation is found between iron stores and serum ferritin concentration in normal humans when liver iron stores were estimated indirectly by quantitative phlebotomy (14), and a less close relationship to bone marrow iron content measured by semiquantitative histochemical means (11). In birds, this relationship has been established between serum ferritin levels and iron stores status from measurements of these parameters in chicken fed with iron deficient and overloaded diets (2), as well as from the consequence of evolution from non-laying hen to laying hen and chicken treated with estrogens (1).

In humans, a relationship between serum ferritin concentration and iron stores is suggestive but has not been demonstrated conclusively since direct measurements of human liver iron stores are difficult. In the present work, iron stores are directly evaluated by means of tissue ferritin content in ducks, before and after treatment with estrogens, in order to extend our knowledge of the relationship between serum ferritin levels and iron stores to other animal species, in which iron mobilization from stores during laying is dramatically exacerbated as compared to that of hen.

Materials and Methods

Animals. For treatment with estrogens, 10 adult male ducks were kept in

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large single cages with water and commercial food *ad libitum*. They were administered intramuscularly 5 mg of beta-estradiol benzoate (Schering) per kg of b.w. at time zero and again 24 h later. Blood samples were taken from wing vein at 24, 48, 72, 96 and 168 h. Five animals were bled out at time zero, five at 96 h and the rest at 168 h. Liver, spleen, kidney, small intestine and heart of each animal were frozen for storage.

For laying studies, because of the shortage of commercial laying duck installations, 20 non-laying hens, 20 to 22 weeks old, and 20 laying hens, 28 weeks old, 86 % egg production (Shaver strain) were used, as well as 20 chickens (Broiler strain) from «Híbridos Americanos» (Valladolid, Spain).

Assays. Ferritin concentration in duck and serum was measured by a RIA method described before (4). Tissue ferritin was determined after quantitative isolation of ferritin by the method of DRYSDALE and MUNRO (5): Tissues were homogenized in 4 volumes of sodium phosphate buffer (0.1 mol/l, pH 7.4) and heated at 70°C for 10 min. The homogenate was cooled to 4°C and centrifuged at 1,500 *g* for 15 min. The supernatant was assayed for ferritin concentration by the RIA method and for ferritin iron by the o-phenanthroline method (7). Serum iron was also measured by the o-phenanthroline method.

Statistical analysis was performed by the t-Student test to compare two situations and by the analysis of variance to compare more than two.

Results

Table I shows serum ferritin and serum iron concentration in two different situations: laying and non-laying hens, as chicken behaves as non-laying hen (10). Serum ferritin was found to be slightly higher in laying than in non-laying hens ($p < 0.005$).

By means of the RIA technique it could be seen that horse spleen ferritin did not produce any inhibitory effect on the affinity between duck liver ferritin and antibodies anti-duck liver ferritin, even at 10 mg/l concentration, whereas hen, pheasant, quail and pigeon serum produced it, although just the inhibitory effect produced by hen serum was not dependent on hen serum dilution for the two dilutions assayed: 1/25 and 1/50. The latter dilution was selected for serum ferritin measurements as providing concentrations below 500 µg/l, which constitutes the upper limit of detection for the used RIA method.

Treatment with estrogens produces in ducks a strong increase on serum iron levels with a maximum at 96 h (fig. 1). At the same time, iron stores become depleted in less than 168 h and serum

Table I. *Serum ferritin (ng/ml) and serum iron (µg/100 ml) in chicken, non-laying hen and laying hen (Mean ± SD).*
Comparison of results was made by the t-Student test between chicken-nonlaying hen and laying-nonlaying hen.

	Chicken	n	Non-laying hen	n	Laying hen	n
Serum ferritin	2.958 ± 837	24	3,175 ± 744 NS	20	3,756 ± 660*	24
Serum iron	136 ± 7	16	147 ± 7 NS	13	720 ± 54*	16

* $P < 0.005$; NS, non-significant.

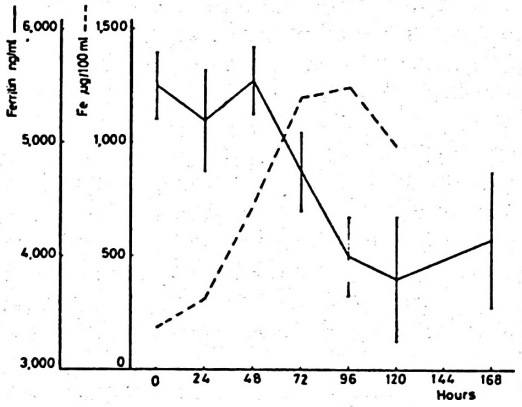

Fig. 1. *Duck serum ferritin and serum iron (Mean ± SD) after treatment with estrogens.*

ferritin decreases significantly with a minimum at 120 h. Table II shows that ferritin content and/or ferritin iron content from all tissues studied can be modified by the effect of estrogens, depending on the physiological activity of each tissue. Iron mobilization from liver is clearly the responsible for serum iron increase, because of its weight and iron content.

Regarding liver, it can be seen that, in spite of the great decrease in iron ferritin and ferritin content itself, the ratio for both from 0 h to 168 h remains nearly constant. Therefore, as serum ferritin decreases, it may be thought that, iron *in vivo*, is mainly delivered from liver to blood by ferritin digestion.

There is no relation between serum iron levels and serum ferritin concentration in ducks, as it had been previously reported in humans (12); on the contrary, serum ferritin seems to reflect the changes in the iron stored represented by the main iron store, the liver (table II).

Discussion

Serum ferritin is normally evaluated with immunoassays based on ferritin extracted from liver or spleen (15). However, in the last years, the validity of results obtained with this kind of immunoassays has been questioned. HAZARD *et al.*.(6) showed that ferritin concentration measured by RIA is greatly affected by the origin of ferritin used as antigen, and the same results were obtained by WORWOOD *et al.* (16). However, more recently, CAVANNA *et al.* (3) have developed a monoclonal antibody against human heart ferritin and have applied it in an immunoradiometric assay (IRMA); their results

Table II. *Ferritin and ferritin iron (µg/g) in heart, kidney, intestine, spleen and liver at 0,96 and 168 hours after treatment of ducks with estrogens (Mean ± SD).* Number of specimens, 5. Comparison of results was made by the t-Student test between 0 h to 96 h and 96 h to 168 h. NS, non-significant.

	0 h		96 h		168 h	
	Ferritin	Ferritin Iron	Ferritin	Ferritin Iron	Ferritin	Ferritin Iron
Heart	42± 8	7.1± 1.7	18± 7**	3.8±0.4**	15± 7 NS	3.2±0.9 NS
Kidney	52±22	8.3± 2.9	83± 8**	9.5±2.5 NS	29±25*	7.7±2.0 NS
Intestine	75±37	4.1± 1.0	90±17 NS	1.9±0.5**	35±19**	2.1±0.6 NS
Spleen	90±19	21.1± 3.6	112±30 NS	5.7±1.3**	115±46 NS	6.2±1.0 NS
Liver	386±95	35.3±10.9	267±45*	21.8±1.4*	159±50*	11.4±3.3**

* $P < 0.05$; ** $P < 0.005$.

show that the more acidic isoferritins account for less than 5 % of total serum ferritin in human, as it had been reported by JONES and WORWOOD (9). This result may probably be extended to ducks because, in spite of the evolutional difference between ducks and humans, it is likely that serum ferritin has the same tissue origin in both species. Therefore, RIA used in the present work to evaluate serum ferritin, although based on the more basic isoferritins, is valid for measuring serum ferritin accurately.

Ferritin content of different tissues has also been measured by the RIA method and the values might be affected by the source of ferritin used as antigen, but absolute values are not so important as the physiological relative variations for the same tissue, which are unaffected by the source of ferritin.

Serum ferritin levels decrease in ducks after estrogenization in a similar way to that observed in chicken by CALVO *et al.* (1). The same authors found that during the evolution from non-laying to laying hens, serum ferritin decreased too (2). In the present work, evidence is presented that contradicts their observation. However, it is likely that serum ferritin decrease found during the passage from non-laying to laying hen responds to a transient situation of iron mobilization to which the body is not trained, while results presented here correspond to two well-defined steady-state situations in which the iron absorbed would satisfy the needs of body without mobilization of iron stores. This observation could be applied to decrease of serum ferritin and tissue ferritin after treatment with estrogens.

SIIMES *et al.* have suggested that ferritin in serum turns over sufficiently rapidly as to account for the transport of most of the iron liberated daily by RE cells (13). In duck and chicken, serum ferritin concentration is several times higher that in humans, therefore, its role as iron carrier should be more credible. In spite of this, the evidence provided by treatment with estrogens contradicts this possible function of serum ferritin in birds, where a third iron carrier is not necessary.

According to MUNRO and LINDER (11), iron mobilization from hepatocyte occurs by digestion. They suggest that masses of cytoplasm, including organelles and ferritin, become engulfed in autophagic vacuoles that become autophagosomes where the citoplasmic constituents undergo digestion and the iron is released into a pool of chelatable iron in the cell sap, easily exchanged with blood. Results presented here strengthen this suggestion.

As in humans (8, 14) and chickens (1, 2), duck serum ferritin reflects iron stores status, although further investigation is needed to establish some type of correlation between them as it has been done in humans (14).

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Resumen

Se ha medido por un método de RIA el contenido de ferritina sérica y tisular en patos estrogenizados, así como la concentración de ferritina sérica en gallinas ponedoras y no ponedoras. Tanto la ferritina sérica como la tisular disminuyen durante la estrogenización al mismo tiempo que la sideremia aumenta. Se ha comprobado la existencia de una relación entre los niveles de ferritina sérica y el estado de los depósitos de hierro.

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