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Capacity of transport of iron in the serum of birds and mammals in «in vitro» tests*

by
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It is well known that iron is transported by the serum forming a complex with transferrin, a protein which belongs to the beta-1-globulin group. This mechanism is universal in all the vertebrates, but in various species of bird it has been demonstrated (8) that there must necessarily exist a powerful auxiliary transport mechanism, for especially in the laying females the values of serum iron are very high. This latter phenomenon was first observed by RAMSAY and CAMPBELL (14) and later a confirmed by PLANAS and CASTRO (5) in the hen and, in the duck and goose, by PLANAS and RECIO (6), who also observed how in these species, and in the laying female, the serum iron is superior to the iron binding capacity of the transferrin.

PLANAS and MARTÍN MATEO (3, 11) have evaluated immunologically the content in conalbumin of the serum of the hen and other species of birds, and the values obtained permit a quantitative explanation of the increase in the total capacity of iron transport to be found in these species. These studies show how the content in serum conalbumin is very much higher in the females — with maximum values in those in the laying period — than in the males.

Studies *in vitro* and *in vivo* carried out on *Gallus domesticus* with Fe-59 and analysis with electrophoresis on paper and immunoelectrophoresis (4) do not permit us to separate the two forms of transport (transferrin and conalbulmin), these proteins behaving identically. The chemical and immunological affinity was already pointed out by WILLIAMS (16) in concrete form, and had previously been suggested by KAMINSKI and DURIEUX (1).

The absence of conalbumin in the serum of mammals determines that all the transport of iron depends on trans-

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ferrin, which is sufficient in view of the physiological characteristics of this group of animals. The values of serum iron and content in transferrin (iron binding capacity) in different mammals has also been studied by us in normal specimens (7) and in comparison with castrated animal (10). The differences with regard to species and sex are very small and, in general, not significant.

In view of the facts so far stated, we have thought it of interest to make a comparative study of the behaviour of the serum of birds and that of mammals when faced with known overloads of iron *in vitro* which may reach and even pass the transport capacity of the transferrin, analysis being made in each case of the iron not fixed by the serum.

Material and Methods

A study has been made of different lots (280 specimens) of serums of chickens and of laying and non-laying hens, as also of 22 serums from mammals belonging to two species (sheep and bull) including both sexes.

The aplication of the different analytical methods utilized required a volume of serum which it was not possible to obtain in the birds individually. This has obliged us to employ generally a mixture of serums from various specimens. In the mammals, on the contrary, it has been possible to carry out the experiments independently on each specimen.

The methods employed in the present work were the following:

1. *Determination of the serum iron and of the iron binding capacity of the serum.* In the various lots of serum employed, or individually in the case of the mammals, an evaluation was made of the content of serum iron and of the iron binding capacity of the transferrin according to the techniques proposed by RAMSAY (12, 13). With these values the unsatured iron binding capacity of each serum was obtained, showing us the real transport capacity of the same.

2. *Addition of overloads of iron.* The method employed for this study was an adaptation of that described by KLEIN (2) for the direct determination of the unsatured iron binding capacity.

In the serums of birds addition was made, in volumes equal to the same, of growing quantities of Fe^{++} (starting from a solution of 1,000 µg Fe^{++}/100 ml, in the form of ammonic ferrous sulphate in HCl 0.002 N) which correspond to final additions of 70, 140, 210, 300, 400 and 500 µg Fe/100 ml of serum. In the mammals the solutivas added were of 100, 200, 300 and 400 µg Fe/100 ml.

After 15 minutes of the serum being in contact with the solution of Fe^{++} in order to ensure the fixation of the metal by the serum, the proteins were precipitated with a solution saturated (80 %) in $SO_4(NH_4)_2$, and after a minimum repose of 6 hours the different test specimens were filtered. Then, following Klein's technique (2), we determined the iron not fixed in equal volumes of the different samples.

3. *Measuring of the radioactivity (Fe-59) in overloads of stable iron.* The behaviour of the serums in the face of growing additions of iron was studied by the presence of radioactivity in the filtrates, mixing a fixed quantity of Fe-59 with different overloads of stable iron.

The procedure followed was partly the same as that described in the preceding section, there being prepared in each lot of serums a growing series of additions of stable iron which in this case was of 35, 70, 110, 150, 200, 250 and 300 µg Fe^{++}/ ml serum. In each tube of one and the same series an equal quantity of Fe-59 was added, which oscillated between 0.2 and 0.5 µc/ml of serum, according to experiment. The Fe-59 comes

from the Radiochemical Centre, Amersham, England.

After 15 minutes of contact between the serum and the stable and the radioactive iron, the proteins were precipitated with a solution saturated in $SO_4(NH_4)_2$. In equal volumes (1/ml) of filtrate, placed in small metal containers, and with desiccation of the samples under an infrared lamp, the radioactivity was measured on a Philips apparatus (electronic counter PW4380, GM tube type 18532 and sample changer PW213-20/00), the geometry being 2π and the distance 2 cm.

Results

In table I we give the results obtained in the different lots of serums of chickens and hens in the addition of growing quantities of iron, with an indication of the values of serum iron and the total binding capacity. We have calculated the relation existing between the quantity of iron added to the serum and that found in the filtrate (non-fixed iron), the following straight lines of regression being obtained:

for the chickens: $Y = 0.426X - 61.0$
for the hens : $Y = 0.11\ X - 7.10$,

X being the µg of Fe added and Y the µg of Fe found in the filtrate.

In figure 1 we indicate these relations graphically, together with the theoretic relation between the two variables, according to the mean value of the unsatured iron binding capacity of the chickens. The theoretical values are obtained by substracting the iron binding values from the sum of serum iron and µg Fe added %.

In table II are shown the radioactivity values obtained in other lots of serums of chickens and hens in the face of growing additions of stable iron, and in figure 2 the different behaviour of the two groups of serums can be clearly appreciated.

The mean data corresponding to the two species of mammals studied are given in table III. In the mean values of serum iron and of iron binding capacity no distinction has been made with regard to sexes. Similary, we have grouped the data concerning the growing additions of iron for both species as their unsatured iron binding capacities are identical.

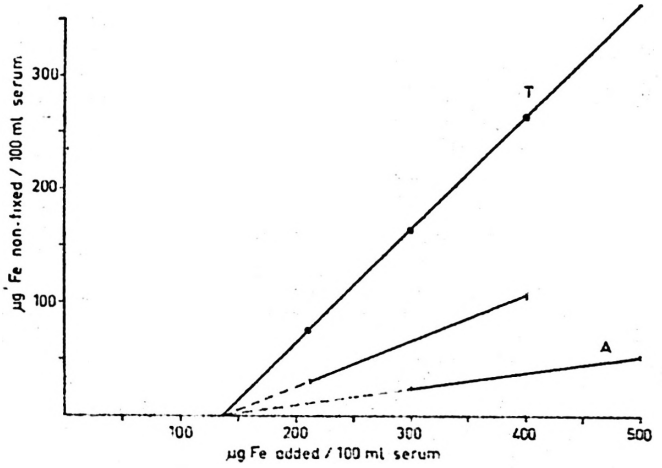

FIG. 1. Regressions lines in hens (A), chickens (B) and theorical relation (T) between the iron added and the iron non-fixed by the serum.

TABLE I

Serum iron (SI), iron-binding capacity (IBC), latent capacity (LC) and data on «in vitro» fixation of iron in hens and chickens.

Lot Sex	N.° specimen	SI µg Fe %	IBC µg Fe %	LC µg Fe %	µg Fe non-fixed per 100 ml serum					
					µg Fe added/100 ml serum					
					70	140	210	300	400	500
Chickens										
I	18	90	240	150	0	0	45	105	140	—
II	24	100	255	155	0	0	25	85	115	—
III	24	120	260	140	0	0	25	50	90	—
IV	24	100	200	100	0	0	10	40	95	—
m ± s		102 ± 6	239 ± 4	137 ± 6						
Hens*										
L. I	18	500	370	—	0	0	0	20	25	35
L. II	20	530	300	—	0	0	0	20	50	55
NL. III	24	170	263	93	0	0	0	20	40	45
NL. IV	16	140	250	110	0	0	0	25	50	60

* L = laying ; NL = non-laying.

TABLE II

Serum iron (SI), iron-binding capacity (IBC) with data on radiactivity found in 1 ml protein-free filtrate after addition to the serum the same doses of Fe-59 and increasing amounts of stable iron.

Lot Sex	N.° specimen	SI µg Fe %	IBC µg Fe %	µc Fe^{59}/ ml serum	Counts/min/ml filtrate						
					µg Fe stable added/100 ml serum						
					35	70	110	150	200	250	300
Chickens											
I	24	110	220	0.32	28	66	175	224	200	—	—
II	18	150	280	0.30	30	60	95	173	137	—	—
III	18	140	260	0.30	43	77	113	218	141	124	—
IV	15	120	260	0.27	40	70	136	188	221	241	—
V	12	130	225	0.23	53	64	82	164	127	—	—
Hens											
L. I	1	530	290	0.20	0	5	7	10	6	6	—
L. II	10	500	300	0.21	0	6	10	11	12	7	12
L. III	8	460	290	0.50	12	21	18	17	25	18	18
NL. IV	4	140	250	0.23	0	20	23	26	26	26	32
NL. V	1	150	270	0.50	9	18	16	23	18	9	5

L = laying ; NL = non-laying.

TABLE III

Serum iron (SI), iron-binding capacity (IBC), latent capacity (LC) and data on the fixation «in vitro» by the sera of lambs and bulls.

Specie	N.°	SI µg Fe %	IBC µg Fe %	LC µg Fe %	µg Fe non-fixed per 100 ml serum			
					µg Fe added per 100 ml serum			
					100	200	300	400
Lambs	10	162 ± 11	328 ± 9	166	0	33	115	190
Bulls	12	163 ± 7	327 ± 13	165	0	53	133	215
m ± s					0	43 ± 7	124 ± 12	202 ± 14

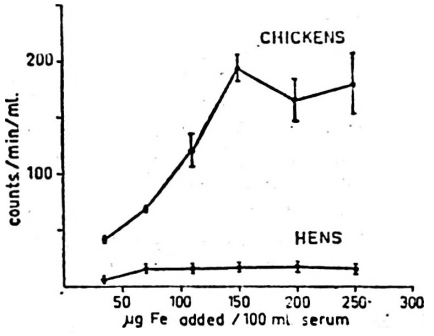

FIG. 2. Variation in the radiactivity in the protein-free serum filtrate after addition of the same amount of Fe-59 and increasing concentrations of stable iron.

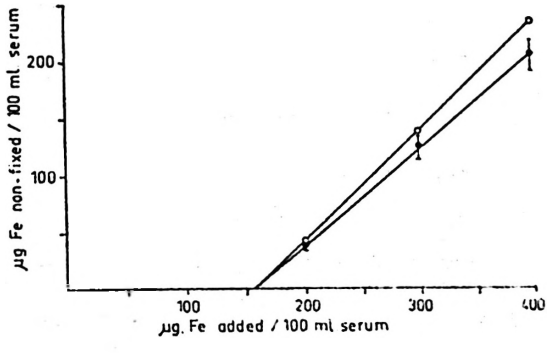

FIG. 3. Relation between the iron added and the iron non-fixed by the serum of mammals (●) against the theorical values (O).

Thus, in figure 3, there is a graphic expression of the experimental values obtained in the two species of mammals vis-a-vis the theoretical values foreseen.

Discussion

The results obtained with the addition of iron *in vitro* show us clearly and directly how there exist different mechanisms for the transport of serum iron in the birds and in the mammals studied. The different behaviour of the serum in the face of additions of iron is reflected in figures 1 and 3. In the mammals (fig. 3) the values of iron not fixed by the serum are very close to the theoretical values in accordance with the unsatured iron binding capacity of transferrin. On the other haud, in the serums of chicken and hen (fig. 1) these values are far removed from those which correspond, theoretically, to transferrin, and show an appreciably increased fixation capacity for iron.

In the hens the divergence from the theoretic is even more pronounced than in the chickens, wich is more evident in the *in vitro* tests with the addition of Fe-59 and stable iron (fig. 2).

All these results are in perfect agreement with the studies previously carried out (3, 5, 6, 8, 9, 11, 15) and constitute

an experimental proof of the hypothesis then suggested that in birds must exist a second mechanism for the transport of iron, and one mechanism of iron transport and the of capital importance, especially in the females, for the laying phenomenon implies a great activation of the metabolism of the iron.

We have observed (5, 6, 8) how in laying birds the serum iron is greater than the iron binding capacity, thanks to a particularity of Ramsay's method (13) for determining this latter. We have seen that the CO_3Mg which is added to absorb the excess iron only captures the free iron or that fixed to the conalbumin, as we have confirmed directly in different concentrations of the conalbumin-Fe complex (3). That is why the iron binding capacity of the iron according to this method of Ramsay has reference only to the transport capacity of the transferrin, which forms with Fe a much more stable complex.

Mereover, it has later been proved in the hen (15), by three different methods, that the unsatured iron binding capacity of transferrin is null in laying specimens. Similarly, the conalbumin values found by immunological evaluation in the serum of the species *Gallus domesticus* (3) show us how its content is less in the chickens, while in the hens it increases considerably in relation with the laying period, when this protein fraction comes to represent about 70 % of the total transport capacity of the serum. These data are especially valuable in explaining the behaviour of the serum of birds in face of growing additions of iron *in vitro* (tables I and II; figs. 1 and 2).

It is, therefore, we believe that in birds there are two physiological mechanims for the transport of iron by the serum, represented by transferrin and conalbumin.

In mammals (7) there is never any inversion of the values of serum iron and of iron binding capacity determined with Ramsay's methods (12, 13), nor do we know of the presence of a protein fraction equivalent to conalbumin, so that transferrin is considered the only behaviour of the serum *in vitro* confirms this supposition (table III and fig. 3).

Summary

A study has been made *in vitro* of the capacity of transport of iron in serum of chickens and hens, and of two species of mammals (sheep and bull).

It has been demonstrated that the behaviour of these serums in the face of growing additions of iron is clearly different. In the mammals, once the unsatured iron binding capacity of the transferrin has been passed values of nonfixed iron apear which are very close to those theoretically foreseen. In the birds, on the contrary, the serums can always fix a quantity of iron which is much higher than that corresponding to transferrin.

The experimental results obtained constitute a direct proof of the intervention in the serum of birds of a second protein mechanism for the transport of iron, which is in addition to the usual in all vertebrates, constituted by transferrin.

From other previously published papers, we consider to the conalbumin as this second iron transport system.

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