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Blood Iron Metabolism in Fowl and Rabbit *

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Fe-59 plasma clearance, the turnover values and the reappearance in the red cells have been analysed on four lots of animals: 16 laying hens; 7 two month old pullets; 7 two month old cockerels; and 11 buck rabbits.

It has been observed that: *a*) plasma clearance is different between the lots and that the fowl always offer the existence of two rates for this phenomenon; *b*) the turnover values in the fowl are superior to those of the rabbits, especially those determined during the laying period; *c*) the differences between fowl and rabbits are also apparent in the red cell reappearance of the Fe-59, with reference to the shape of the curve and the duration. In the fowl, fluctuations are observed which coincide with the life span of the red cells. Laying birds always show lower radioactivity levels; *d*) the distribution of the radioactivity in the eggs shows variations in intensity which are related with the variations of the blood Fe-59.

With these results to hand, the peculiar iron metabolism in fowls is also evident in *in vivo* studies, as a result of the egg laying which affects not only the transport mechanism in the plasma but, especially, the distribution and the use of the iron in the haemoglobin synthesis and in the egg production.

Plasma iron transport capacity in mammals and fowl has already been discussed in previous publications (9, 10). The difference between these groups of animals has been shown, since *in vitro* studies show that fowl plasma can transport an iron values in excess of the expected level in accordance with the transferrin content.

The administration of Fe-59 and the study of plasma clearance and determination of the corresponding turnover is a technique used to appreciate the characteristics of iron metabolism. These studies have been performed on man, dogs and rabbits (5, 6, 8).

With reference to fowl, the behaviour of Fe-59 has been studied in the hen by HALKETT *et al.* (4), by analysing radioactivity in laying in relation to globular destruction and plasma iron. RAMSAY (13) has taken the same species to study iron take up and haemoglobin synthesis in the erythrocytes, with the aid of radioactive iron,

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whilst KLEIN (7) has analysed the incorporation into the erythrocytes in ducks.

This paper gives a comparative analysis for hens and rabbits of Fe-59 plasma clearance, the turnover values and reappearance in the red cells, in order to analyse the transport mechanisms and understand certain aspects of iron metabolism which would enable the behaviour differences between fowl and mammals to be evaluated.

Materials and Methods

The following lots of animals have been studied: 1st, laying hens (16 specimens); 2nd, two month old pullets (7 specimens); 3rd, two month old cockerels (7 specimens); and 4th, buck rabbits (11 specimens).

Fe-59 was incorporated by incubation for 15 minutes into 2-3 ml of autologous plasma and then injected endovenously in doses varying between 0.5 and 5 μc/kg. The specific radioactivity of the different samples of ferric citrate (Fe-59) used has varied considerably, thereby allowing for very small additions of free iron, in face of experiments with very conspicuous values.

The plasma iron and the total iron binding capacity in each specimen has been determined according to RAMSAY (11, 12) which has allowed to see accurately in each case if the addition of free iron exceeded the unsaturated iron binding capacity of the plasma.

After the endovenous injection of Fe-59, heparinised blood samples were taken at 3, 15, 30, 60, 120 and 180 minutes. The radioactivity in 1 ml of plasma has been measured in a well type scintillation counter.

The plasma iron turnover has been calculated according to the indications of BOTHWELL and FINCH (2). For these calculations we have taken the T-1/2 (time taken for radioiron to reach half concentration) appearing in the first clearance curve.

Blood samples have been taken at 7.30, 24 and 48 hours after injection and the radioactivity of the plasma and red cells has been compared on like volumes. As from tris time, blood samples are taken every week for a period of 3-5 months. This study has been performed on one group of animals from each lot: i.e.: 8 laying hens; 4 pullets; 6 cockerels and 4 rabbits. In the case of the laying hens, the radioactivity in the eggs has also been measured.

Results

Table I gives the values obtained from the different animals studied. Also the average values and the resulting dispersions in each lot are given. The wide variability of results justifies the inclusion of the individual values.

Figure 1 gives the average plasma clearance lines in the lots studied. It is obvious how all the fowl lots show two straight lines of plasma clearance, irrespectively of whether the iron addition ex-

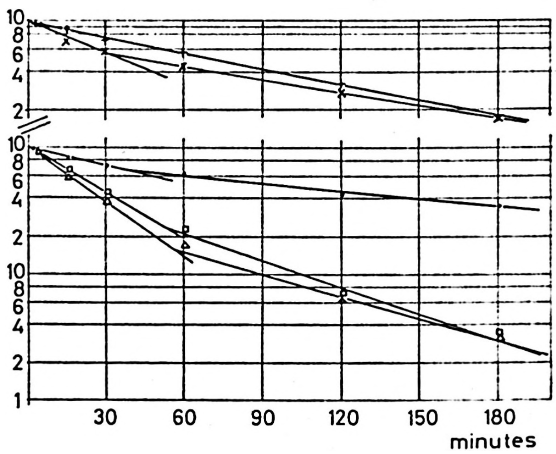

Fig. 1. *Clearance rates in the rabbits with iron lower (+) or higer (×) than their UIBC and in the fowl.*

●, laying hens; □, cockerels; △, pullets.

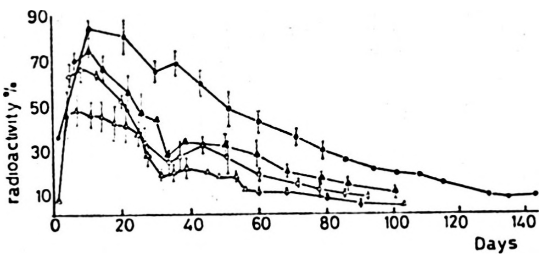

Fig. 2. *Radioactivity (Fe-59) in blood in the different animals analyzed.*

●, rabbits; ○, cockerels; △, laying hens; ▲, pullets.

ceeds or not the plasma transport capacity. On the contrary, in the case of the rabbits, two clearance curves appear only when the plasma unsaturated iron binding capacity is exceeded (rabbits n.° 1 to 7).

Plasma clearance in the fowl is total between 9 and 24 hours after injection and the reappearance in the red cells as haemoglobin iron already is evident at 24 hours (6 %) with the high values (30 %) being reached at 48 hours.

The appearance of the Fe-59 in the red cells, as labelled haemoglobin, and its maintainance over the time, shows a characteristic distribution (Fig. 2) where notable differences between fowl and mammals are to be seen.

Egg radioactivity has been analysed in 8 laying specimens. Figure 3 shows the distribution of radioactivity over the laying period in 4 specimens and this may be considered as typical.

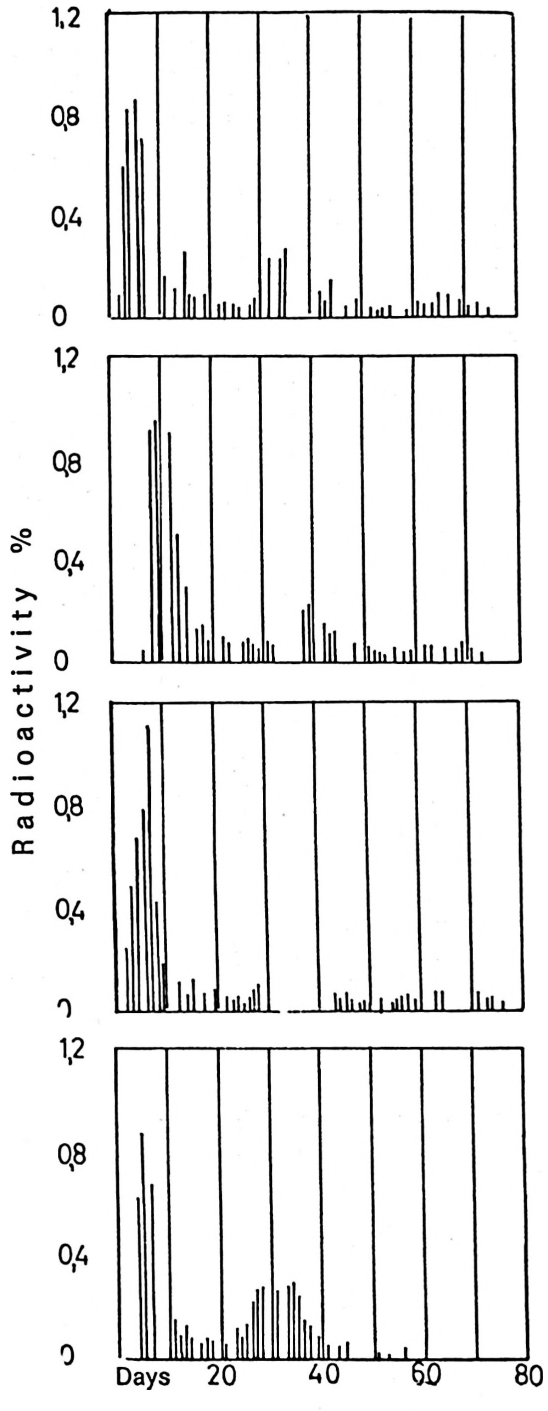

Fig. 3. *Fe-59 content in the eggs from some hens, expressed in porcentages of the total amount injected.*

Discussion

PLASMA CLEARANCE. — The injected radioactive iron disappears from the plasma exponentially, so that radioactive values are nil as from 8 to 14 hours after injection.

The plasma clearance values are different in the animal lots studied (Fig. 1) and the different clearance rate is shown in the value of T-1/2.

Table I. *The individual values of the specimens.*

Species and specimens	Weight Kg	Plasma Iron µg Fe %	TIBC * µg Fe %	T-½ (minutes)	Turnover µg Fe/100 ml 24 h.	Dose µc/Kg	Dose µg Fe %	Volume (ml) Blood	Volume (ml) Plasma	Hematocrit.	Clearance values (% Radioactivity) Minutes 3	15	30	60	120	180
Laying Hens																
1	3	490	176	85	3810	1.3	0.06	131	89	32	93	91	79	61	48	39
2	1.3	590	495	50	7600	1.8	0.003	89	62	31	96	80	68	60	39	32
3	3	590	465	90	4000	0.9	0.003	128	86	33	98	89	80	76	52	46
4	3	645	352	95	4550	0.1	0.003	61	41	33	96	90	81	72	45	37
5	3	530	345	52	7930	0.5	0.006	118	80	32	93	81	67	60	45	40
6	1.5	625	390	43	10020	1.6	0.12	96	68	29	93	83	63	52	36	29
7	3	415	367	52	5250	0.8	0.12	112	74	34	96	79	67	52	38	30
8	4.1	615	525	58	7750	0.5	0.12	143	100	30	97	81	77	65	44	28
9	2.8	450	645	115	2440	0.9	400	191	120	37	94	93	92	72	64	49
10	2.2	450	472	70	3836	1.4	373	152	100	34	95	82	74	67	62	46
11	3.6	450	322	67	4140	1.3	530	139	90	34	95	88	70	56	42	35
12	3.7	315	350	80	2210	1.4	545	143	88	38	98	93	84	59	35	24
13	1.7	475	390	52	6000	0.9	93	108	71	34	96	76	69	56	41	36
14	2	490	495	50	6400	0.9	94	76	49	35	97	80	67	49	36	28
15	1.6	490	380	48	7150	2.8	485	94	66	30	95	81	65	50	41	29
16	3.1	450	390	57	5420	1.5	580	153	104	32	98	83	74	57	39	25
m ± σ		504±89	410±106	66±21	5532±2167			121±33	80±21	33±2	96±2	84±5	73±8	60±8	44±8	34±8
Cockerels																
17	2.5	110	175	27	2740	2.4	50	138	95	31	91	61	46	30	14	9
18	2.4	80	175	22	2650	3.0	66	100	68	33	92	63	40	18	7	3
19	2.1	115	157	20	3840	3.4	50	133	93	30	89	59	36	14	3	1
20	1.7	75	128	32	1860	3.5	36	111	87	22	91	73	49	28	5	2
21	1.4	105	120	20	3690	4.8	36	79	57	28	90	62	32	13	2	1
22	1.4	115	113	35	2440	5.1	40	71	53	26	91	73	54	28	6	2
23	1.3	130	150	30	2970	5.4	50	96	66	31	93	68	51	33	13	6
m ± σ		104±20	145±25	26±6	2884±694			104±25	74±17	29±4	91±1	66±6	44±8	23±8	7±5	3±3

Pullets																
24	1.4	40	232	18	1580	1.2	0.1	67	49	27	88	55	29	9	2	0
25	1.2	75	412	13	3860	0.98	0.07	67	45	32	86	43	21	7	3	2
26	0.9	115	382	24	3250	0.65	0.04	87	61	30	92	67	43	18	8	4
27	1.1	100	360	22	3200	0.55	0.04	104	74	29	82	64	38	15	6	4
28	1.5	75	202	30	1710	4.4	48	83	58	31	92	71	52	30	15	9
29	1.7	100	172	25	2880	4.1	48	98	69	29	94	62	44	21	7	3
30	1.6	112	150	19	4090	4.3	43	82	59	28	86	62	36	13	3	1
m ± σ		88±26	273±109	22±5	2939±975			84±14	59±10	29±2	89±4	61±9	38±10	16±7	6±4	3±3
Rabbits																
1	2.4	175	450	38	2440	1.76	540	157	85	46	93	77	57	32	26	19
2	2.5	105	300	30	2225	1.68	270	137	88	36	97	65	50	35	16	12
3	2.3	205	322	40	2820	2.6	362	143	79	45	91	76	59	41	28	17
4	2.1	150	352	38	2190	3.6	120	234	131	44	95	60	60	52	39	26
5	1.5	200	260	35	3480	3.0	540	109	68	38	95	64	57	48	23	13
6	0.8	235	450	35	3120	8.8	290	79	49	38	98	70	57	48	29	22
7	0.8	75	202	33	1333	8.8	290	94	56	41	94	68	56	45	28	14
m ± σ		164±57	334±93	36±3	2515±705			136±51	79±27	41±4	95±2	69±6	57±3	43±7	27±7	18±5
8	1.0	215	382	63	1730	1.5	0.09	112	57	49	98	95	73	58	22	16
9	1.1	250	260	85	1850	0.7	0.05	124	70	44	95	88	77	65	40	21
10	1.2	205	268	58	1720	0.7	0.05	109	64	41	95	75	65	50	24	13
11	1.1	250	442	70	1950	0.7	0.03	133	82	39	94	85	70	53	35	18
m ± σ		230±23	337±90	69±12	1813±80			119±11	68±11	44±4	96±2	86±8	71±3	57±7	30±9	17±3

* TIBC = Total iron binding capacity.

In man, the value of T-1/2 is 85 ± 25 minutes and in normal individuals varies between 60 and 107 minutes (6), whilst dogs give values between 60 and 145 minutes and rabbits give 70-80 minutes (5). In this present study, the rabbit group (n.° 8 to 11) gives values varying between 58 and 85 minutes, in laying hens the average is 66 minutes, whilst in the young specimens, both sexes give 21-26 minutes. The difference between these lots is statistically significant and clearly marks a modification of iron metabolism with laying, whinch is reflected also in the higher plasma iron content and in the turnover increase.

The study of the different clearance curves in fowl shows that all have changes in the clearance rate, expressed in a change in the slope which takes place from 30 to 60 minutes after injection. Multiexponential rates have been shown in rats (T-1/2: 1.2 and 11.8 hours) and in man (T-1/2: 79 minutes and 2.5 days respectively) resulting from a balance between the plasma iron and the plasma storage (8). HOSAIN and FINCH (5) likewise show how, in general, the existence of several exponential curves must be explained by the return of the iron to the plasma pool.

In rabbits is evident a double clearance curve when the plasma unsaturate iron binding capacity (UIBC) was exceeded (rabbits n.° 1 to 7). On the other hand, where the UIBC was not exceeded (rabbits n.° 8 to 11) show only one rate.

The difference between the rabbits and the fowl, presenting always the last ones two clearance rates, should be explain for two reasons: the return of iron to the plasma pool or the existence in the fowl of a double plasma iron transport as has been shown by ALI and RAMSAY (1). Perhaps is more plausible the second explanation in view of the short time in which appear the change of the slope.

PLASMA IRON TURNOVER. — Plasma iron turnover has been determined in man (2) with normal values of 600-650 μg Fe/100 ml blood/24 hours, which means that in a person weighing some 70 Kg plasma pool (3-4 mg) is turned over about 12 times a day. This a point of clinical value since it provides information on the haematopoietic function.

There is no reference available to this in animals. The turnover value in the rabbit (Table I) shows some variation, but in the tot with only one exponential curve, it has lower, more uniform values, but all of them are more than double those observed in man.

In the case of the animals with two exponential curves for plasma clearance, the turnover values must be taken as merely aproximate, by shortcoming, since all of them been calculated with the T-1/2 values corresponding to the first curve.

In spite of this, the high value in fowl, increased in the period of laying, is notorius. The high turnover values in cockerels and pullets, with low plasma iron levels (75-120 μg Fe %) are obtained as a result of the very high disappearance rate (T-1/2, 20-26 minutes) equivalent in man to ferropoenic anemias.

In laying hens, the clearance rate diminishes (T-1/2 = 66 minutes) but the plasma iron rises (450-680 μg Fe %) where by the turnover is greatly increased.

The comparison of the absolute values of plasma iron in the plasma pool with respect to the daily turnover (Table II) show how in fowl the contents of the plasma pool is renewed daily over 40 times, in face of the 10-14 times in rabbits and man. In the laying hen, the plasma iron increases 7 to 8 times above the level of the young specimens and the daily interchange is triplicated in spite of increasing the T-1/2, with a descent in the renewal of the plasma pool to 16 times a day. There is a close relationship between the plasma iron levels and its disappearan-

Table II. *Comparation in several species between the total plasma iron, the total iron interchanged and the dayly renovation number of the total plasma iron pool.*

Species	Total iron in the plasma pool mg	Iron interchanged in the plasma pool in 24 hours mg	Dayly renovation number of the total plasma iron pool
Laying hens	0.40	6.69	16.7
Pullets	0.05	2.08	41.6
Cockerels	0.07	2.98	42.6
Rabbits	0.15	2.17	14.3
Man	3.50	35.00	10.0

ce rates. This has been evidenced in man in different normal and pathological states (2).

GLOBULAR REAPPEARANCE OF FE-59. — In fowl, 9 hours after endevenous injection of Fe-59, the plasma content is about 6-10 % whilst it is nil in the red cells. At 24 hours, the plasma radioactivity has disappeared completely and it has reached 6 % in the red cells and at 48 hours this value has risen to 30 % approximately and continues to increase in following days. The rate of globular reappearance of the Fe-59 differs between fowl and rabbits (Fig. 2) and there is a further difference within the fowl as far as laying birds are concerned.

In fowl the globular reappearance is very quick and reaches a maximum level between the 5th and 10th days, rising to over 70 % in the young specimens and to only 50 % in the laying birds. As from this time, there is a drop in the red cell radioactivity with two clearly definid minima around the 32nd and the 60-65th day. Laying hens follow the same rhythm but their radioactivity levels are lower and show a plateau between the 5th to the 20th days approximately. These results agree exactly with those observed by HALKETT *et al.* (4) in the same species.

The inflection points represent the destruction of the red cells and the incorporation of the freed Fe-59 to recently formed red cells. The average life span of the red cells is 24 days in the hen (4) and the destruction of the old cells frees the iron which increases the plasma pool.

The globular reappearance curve, specific for laying birds, is explained by the elimination of iron in the lay, details of which will be given in a further section.

In rabbits, the globular Fe-59 shows a different distribution, specially because of the slowness of its disappearance, since the levels are 8 to 10 % after 130-140 days, when these levels are reached at 90 days by the young fowl and at 60 days in the laying birds.

The average life span of the erythrocytes in rabbits is 55 days approximately (3). which implies a slower haemoglobin iron renewal cycle.

RADIOACTIVITY IN THE EGGS. — The appearance of the Fe-59 (Fig. 3) in the eggs is very rapid, in such a way that the maximum radioactivity values are to be seen between the 5th and 10th days and later on a further two maximum levels are to be observed, one between the 25th and 40th days and the other between the 60th and 70th days. According to HALKETT *et al.* (4), the maxima are reached on the 4th, 30th and 59th days, the first, being the intensest, comprises 38 % of the dose injected, whilst the other two are 10 % and 3 % respectively. These authors indicate that the ovary takes the iron direct from the plasma pool, since it has no storage system and, therefore, depends on the plasma iron levels.

The Fe-59 is to be found exclusively in the yolk and the figures given in Figure 3 correspond to the percentages of the total amount injected, obtained by measuring the whole egg. The measurement of the yolk radioactive content, with identical geometry, shows an increase of 10 to 40%.

The total Fe-59 loss in laying has been measured for 8 specimens and represents between 3.58 % and 7.58 % of the injected dose, with a great variability, since it depends on the intensity of the lay. The overall values of the lay found are very low, since even when measuring the yolk radioactivity, it would get 10 % when it is 38 % in the first lay peak under the conditions expressed by HALKETT *et al.* (4).

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