قسم : الكيمياء الحيوية - كلية الطب - جامعة أسيوط.
رئيس القسم : أ. د. / وفاء أحمد حماد .

حركة أيون الحديد فى جسم الفئران البيضاء

أحمد نصار ، محمد صلاح ، محمد عبدالله

يشتمل هذا البحث على السلوك الديناميكى لايون الحديد فى جسم الفئران البيضاء ولقد اظهر البحث السلوك مستعينا بما سبق للعلماء من وضع نموذج رياضى ليصف هذه الحركة بالنسبة لحديد جسم الانسان ، حيث ان البحث قد نجح فى استخدام هذا النموذج وأستعان بالطرق الرياضية الخاصة بالتحليل الهندسى اللازم للمعطيات المستنتجة من استخدام طريقة افتقاء الأثر الأشعاعى فكان متاكملا اذ أنه استظهر بالأرقام طبيعة البحيرات الابضية التى يتمثل فيهنا هذا الايون الهام كما استظهر بالارقام معدلات ظهور الحديد فى الكرات الحمراء وكذلك زمن تصنيع الهيموجلوبين فى الكرات الغير مكتملة النمو كما أعطى أيضا عمر الكرات البالغة النمو وممما هو جدير بالذكر ذلك التقدير لصافى معدل هجرة هذا الايون من بلازما الدم الى أماكن التصنيع من جهة وأماكن التخزين وأعضائه من جهة أخرى .

واستنتاجا يكون البحث قد أعطى أطارا شاملا لطبيعة توزيع وحركة هذا الايون فى جسم تلك الحيوانات التى تستخدم بكثرة بالغة فى التجارب الحيوية لمعرفة تأثير العديد من المركبات التى يتعامل معها الانسان كما أنه من جهة المعرفة يكون البحث قد رسم الى حد بعيد الأيض الحيوى لهذا الحديد فى جسم الحيوان المذكور .

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IRON-KINETICS IN ALBINO RATS

(With One Table and 5 Figures)

By

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SUMMARY

A mathematical model of iron kinetics derived by POLLYCOVE and MORTIMER (1961) was successfully used on normal adult rats. R.B.C. iron turnover number, mean delay of iron in red marrow for haemoglobinization, and the R.B.C. life span were determined. Complete iron exchange between the main plasma iron pool, and the storage compartments was detected. The findings elucidated the complex equilibrium between the plasma iron and the various iron pools, namely the erythrocytic pool, itself comprising almost equal labile and fixed compartments and the storage pool. The equilibrium shows that the iron actually deposited in the storage pool is little (97 µg Fe/day) compaired to the erythrocytic iron turnover (410 µg Fe/day).

REVIEW

Most of the work published on iron kinetics and metabolism for the interpretations of some pathogenic conditions dealt with human subjects. Models were proposed - POLLYCOVE and MORTIMER, (1961) and LARCE et al.,(1963) and verified by experiments which elucidated the physiology of the systems concerned. The present work described these kinetics in rats by using POLLYCOVE'S model and MOSER and EMERSON'S (1964) mathematical analysis of the plasma radioactivity disappearance curve.

METHODS

The animals were given ^{59}Fe as feric citrate (prepared for injection derived from Amersham's Radiochemical Centre, England, specific activity 20Ci/9). Doses ranged from 5 to 20 UCi, i.e. the injected iron did not exceed 1 Ug Fe which is very much less than the lower limit of the iron-binding capacity (determined previously in a population of 30 rats of both sexes).

Iron content in circulating red blood cells was determined by the cyanmethaemoglobin method (HAROLD VARLEY 1969).

Total plasma iron content was estimated by the method of $\alpha - \alpha'$ dipyridl method (RAMSAY 1958).

Organs taken for counting radioactivity were spleen, liver and kidneys (storage sites), skull and bones containing red marrow (erythropoietic sites).

Counting was done on plasma samples and circulating red blood cells washed several times with saline.

RESULTS

Haematocrit, Total blood volume, Plasma volume, Packed red cell volume, Total iron content in plasma and Total haemoglobin iron in peripheral blood cells are presented in Table 1.

Disappearance of plasma radioactive adminstered dose (% A.D) presented in Fig. 1. The disappearance during the first few hours is presented by a line at the apex of the figure. Let this be called the disappearance time $t_{\frac{1}{2}}$ = 3.6 hours can be used as a special parameter for these animals.

On the other hand the appearance of this activity in circulating red blood cells is presented in Fig. 2. The maximum activeity (F) accumulated (80% A.D.) was at about the fifth day, on which the plasma disappearance activity (Fig. 1) reached its constant remaining value (1% A.D.). The solid curve in Fig. 2 is called N-curve and the fraction (F) the N- maximum and the latter is used in the calculation of the mean effective erythron haemoglobinization time (M.E.E.H.T.). Another dotted curve F-curve is drawn -from the kinetic equations of consecutive reactions (ROBERTS, 1977)- presenting the radioiron accumulated in the compartement F for which irreversible fixation of iron into haem inside mature erythrons.

Fig. 3 presents the movement of radioiron in the bones that containing red marrow. Bones, Skull and ribs accumulate the radioiron in the same manner, i.e. they are similary in the function for haemoglobin synthesis. The individual percentages reach the maximum during the first two days and then the activity leaves these erythropoietic sites after about another two days. This indicates that the activity remains there (after complete accumulation) for about one day. The last remaining % A.D. in these sites remains constant throughout the course of investigation, and is about 5.5% A.D., half of which is captured in the bones and the other is divided in similar Quantities between the skull and the ribs.

Distribution and movement of radioiron in liver, spleen and kidneys is illustrated in Fig. 4. The remaining activity in the liver during the course of the study is about 12% A.D. which is equal to the summation of activity remaining in the spleen (2.5), the kidneys (2) and the bone-red marrow (5.5%) A.D. i.e. the stored iron in the liver is more or less half the vaue of the total stored iron in the white rat's body.

From the mathematical analysis of the plasma radioiron disappearance curve (Fig. 1) by the methods of MOSER and EMERSON (1964), the following parameters were derived:

I- The intercompartmental rate constants α 1 - 2' α 2 - 1' α 1 - 5' α 2-3 and α 5 - 6 illustrated in Fig. 1.

II- Daily iron for haemoglobin synthesis or R.B.C. iron turnover number which equals 0.4098 mg Fe/day.

III- Time of red cell survival or the mean erythron life span (M.E.L.S.) equals 22.578 mg/day - 54.36 days , where 22.578 mg is the mean of total iron content in R.B.Cs. (Table 1).

It is clear from Fig. 2 that the time interval between the incorporation of radioiron into red marrow and its appearance in the circualting erthrocytes (M.E.E.H.T.) be deduced from the formula.

$t\frac{1}{5}$ (B) - $t\frac{1}{5}$ (F) ..... (POLLYCOVE and MORTIMER, 1961). where the longest distance between the two curves is within the 20% accumulation ($t\frac{1}{5}$).

or M.E.E.H.T. = $t\frac{1}{5}$ (N) - $t\frac{1}{5}$ (F) = 0.97 day.

From all the previously derived parameters, the red blood series pool size x_3 was easily deduced to be the daily iron entering the pool multiplied by the time consumed for haemoglobinizolion = 0.97x410 µg Fe = 400 µg Fe. Also the miscible storage iron x_{ms} equals 1.493 mg Fe and the storage iron deposited per day is 0.103 mg Fe/day.

All of these parameters are presented diagramatically in Fig. 5.

DISCUSSION

The proposed model of iron kinetics in man deduced by POLLYCOVE and MORTIMER (1961) is successfully used on white albino rats because, the calculated red cell survival for these animals kinetically is in resemblance to those determined by others (OWIN and ORVIS, 1966).

It is clear from figures 1,2,3 and 4 that the disappeared iron from plasma moves to the red marrow of bones, skull and ribs where the marrow synthesizes haemoglobin which appears in the circulating blood erythrocytes. At the same time the plasma radioiron moves to the liver, spleen and kidneys. This means that the radioriron is poured from plasma compartement into two compartments the first for haemoglobinization and the second for storage.

The erythropoietic and storage compartments accumulate the radioiron to levels 42% and 32% A.D. respectively during the first day after intravenous injection.

From the first glance to figures 2 and 3, shows the close relationship betwee the sites of manufacture of haemoglobin and the produced hamoglobin in the circulation. Also we see that the red marrow in bones, skull and ribs conserve about 5% A.D. for storage hemosiderin and ferritin (POLLYCOVE and MORTIMER 1956 and 1958). On the other hand, the contributed activity in the skull is greater than that in the bones although the red marrow mass is small in skull than that in bones. This may be attributed to storage of iron in brain tissues, since we already know that iron is stored as ferritin in the red marrow of the diploe of the skull,then the data explain tehemselves.

Kinetically the iron moves from the main plasma compartement at the rate of 508 µg Fe,the reveraible movement is 98 Ug Fe per day. The net gain for erythropoiesis or red cell iron turnover is 410 µg Fe per day. The mean delay time for haemoglobinization M.E.E.H.T. is about 0.97 day, which leads to precise calculation of the labile (x_2, 445 µg Fe) and irreversible (x_3, 398 µg Fe) erythropoietic iron pools and red cell survival.

The ferro-kinetics between the main plasma pool and the main storage organs as the liver, spleen and kidneys etc. shows that the migration is 629 µg Fe and the reversible feed-back is 581 µg Fe/day, indicating that the net migrated iron from the main plasma to the main storage compartemtn is 97 µg Fe/day. This value of migrated iron is the rate of iron deposited per day as haemosiderin and ferritin which have almost the same value of 103 µg Fe deposited per day. It has been deduced from the analysis that x_5 is the labile storage iron pool with a size of 163 µg. Fe. The kinetics in this aspect indicate that the iron exchange between plasma and storage pool perists in the animals and persists only in certain anaemic humans (POLLYCOVE and MORTIMER, 1961).

From the comparative point of view we can say that the considerable stored iron exchange with plasma is promenant in the case of normal white rats but it is not so in normal humans.

The released amount of activity (E) from storage pool to the plasma is 1% A.D. indicates the continous feed-back mechanism from the stored iron to the plasma pool. The labile stored iron in this manner is called the miscible storage iron which is resulted in this work as 1,493 µg Fe. Assuming that this corresponds to ferritin and using a haemosiderin: ferritin ratio 1 (SHODEN et al.,1953),the total stored iron is approximately 2, 986 µg. Fe.

From Fig.4 we can see that the accumulated radioactivity in the organs 'liver,kidneys and spleen) is released after about 3 days, of injection to half its value, exponentialy. The spleen and kidneys conserve activity at low levels, but the liver of seven times the former. The summation of the activity remaining in the kidneys, spleen and stored iron in the erythropoietic sites of red marrow and brain tissue is about 10.5% A.D. While the remaining activity in the liver is about 11% A.D. The stored iron in the liver is approximately half the value of the Total stored iron in the whole body of these white rats. This finding in white rats is somewhat similar to that in human where Hallgren in 1954, published that the liver in human beings contains about half of Total storage iron. On the other hand kidneys conserve radioactive iron this conclusion is supported by the published data of HAMPTON and MAYERSON et al. (1950) who studied the factors stimulating the production of ferritin by the kidney.

Generally speeking (from Fig. 5) although the rate of movement of plasma iron towards the erythropoietic and storage pools are somewhat similar (508 and 629 µg respectively), the feed bach from the storage to the plasma pool is large with respect to the erythropoietic to the plasma pool, the value for the first being 531 µg Fe compared to 98 µg Fe for the second. This shows that the net gain of iron for erythropoiesis compared to storage is much more significant (410 and 98 µg Fe/day). We can conclude that the white rat is more engaged in erythropoiesis than in storage because the first value is about 4 times the second one. This is somewhat different in case of human beings where the storage is insignificant compared to erythropoiesis, except in certain cases of anaemias (POLLYCOVE and MORTIMER,1961); but both species are alike in the sense that the body is engaged more in erythropoiesis than in storage.

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TABLE (1)

No.	Sex	W. (g)	H (Vo%)	Bl.V. (ml.)	P.V. (ml.)	R.C.V. (ml.)	P.Fe (Ug.) x_1	R.C.Fe (mg.) x_4
40	♂	208	45.5	19.72	10.75	8.97	94.92	32.52
35	♂	200	45.6	18.14	9.87	8.27	80.50	23.54
34	♂	170	43.0	15.78	8.98	6.80	88.26	23.72
39	♂	210	46.6	20.03	10.70	9.33	10.89	33.56
38	♂	206	48.0	18.67	9.71	8.96	78.84	25.40
50	♀	107	38.1	10.23	6.24	3.99	65.02	15.46
48	♀	105	36.4	9.45	6.01	3.44	89.31	24.36
37	♂	245	53.0	18.01	8.46	9.55	131.13	33.84
46	♀	85	43.2	9.06	5.15	3.91	57.06	12.96
47	♀	93	43.8	9.11	5.12	3.99	70.83	12.76
30	♂	180	42.6	16.87	9.68	7.19	130.68	26.54
15	♂	190	34.0	19.00	12.54	6.46	85.65	30.88
16	♂	175	46.0	15.74	8.50	7.24	112.06	27.94
29	♀	207	40.3	17.91	10.28	7.63	104.34	27.44
6	♀	200	41.0	18.81	11.10	7.71	123.88	27.26
7	♀	110	37.0	9.40	5.92	3.48	44.40	12.22
21	♀	215	45.1	19.03	10.54	8.58	48.43	29.98
5	♂	130	44.4	14.00	7.78	6.22	60.83	21.38
8	♂	150	40.0	14.13	8.48	5.65	78.61	19.06
9	♂	120	38.6	11.05	6.78	4.27	54.78	15.22
17	♂	102	50.0	9.40	4.70	4.70	40.66	12.72
18	♂	135	47.4	12.03	6.33	5.70	84.04	19.84
4	♀	155	85.6	13.18	8.49	4.69	66.90	18.44
14	♂	180	40.0	15.11	9.07	6.04	100.22	25.30
19	♀	100	38.7	9.00	5.52	3.48	41.12	13.48
20	♀	120	43.6	9.84	4.55	4.29	63.83	16.00

W. = weight of the rat in gramms.

H. = haematocrit value.

Bl.V. = total blood volume, P.V. = plasma volume and

P.C.V.= packed red cell volume.

P.Fe. = total plasma iron content in Ug.

R.C.Fe= total red cell iron in mg.

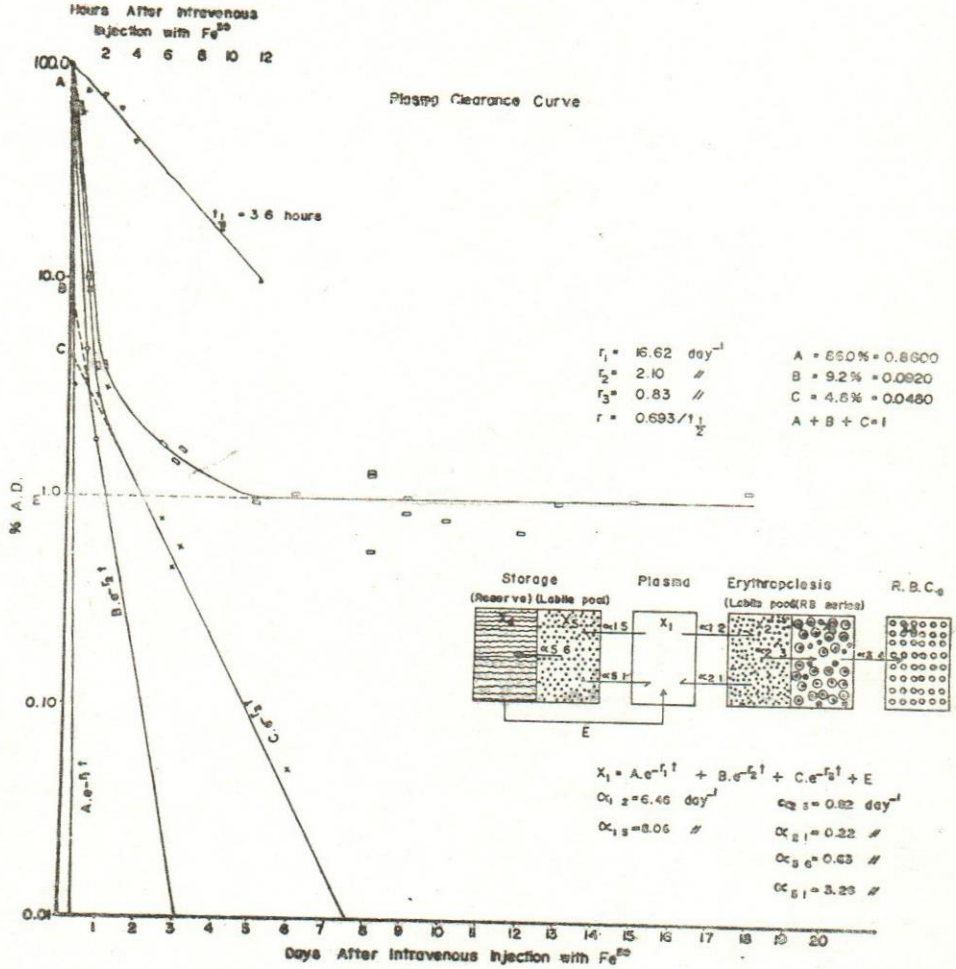

Hours After Intravenous Injection with Fe^{59}
2 4 6 8 10 12
Plasma Clearance Curve
100.0
10.0
1.0
0.10
0.01
% A.D.
A
B
C
$t_{\frac{1}{2}}$ = 3.6 hours
r_1 = 16.62 day^{-1}
r_2 = 2.10 //
r_3 = 0.83 //
$r = 0.693/t_{\frac{1}{2}}$
A = 86.0% = 0.8600
B = 9.2% = 0.0920
C = 4.8% = 0.0480
A + B + C = 1
$A.e^{-r_1 t}$
$B.e^{-r_2 t}$
$C.e^{-r_3 t}$
Storage
(Reserve) (Labile pool)
Plasma
Erythropoiesis
(Labile pool(RB series)
R. B. C_s
X_1
X_2
X_3
E
$X_1 = A.e^{-r_1 t} + B.e^{-r_2 t} + C.e^{-r_3 t} + E$
$\alpha_{1,2}$ = 6.46 day^{-1}
$\alpha_{1,3}$ = 8.06 //
$\alpha_{2,3}$ = 0.82 day^{-1}
$\alpha_{2,1}$ = 0.22 //
$\alpha_{3,6}$ = 0.63 //
$\alpha_{3,1}$ = 3.23 //
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days After Intravenous Injection with Fe^{59}

Fig. 1

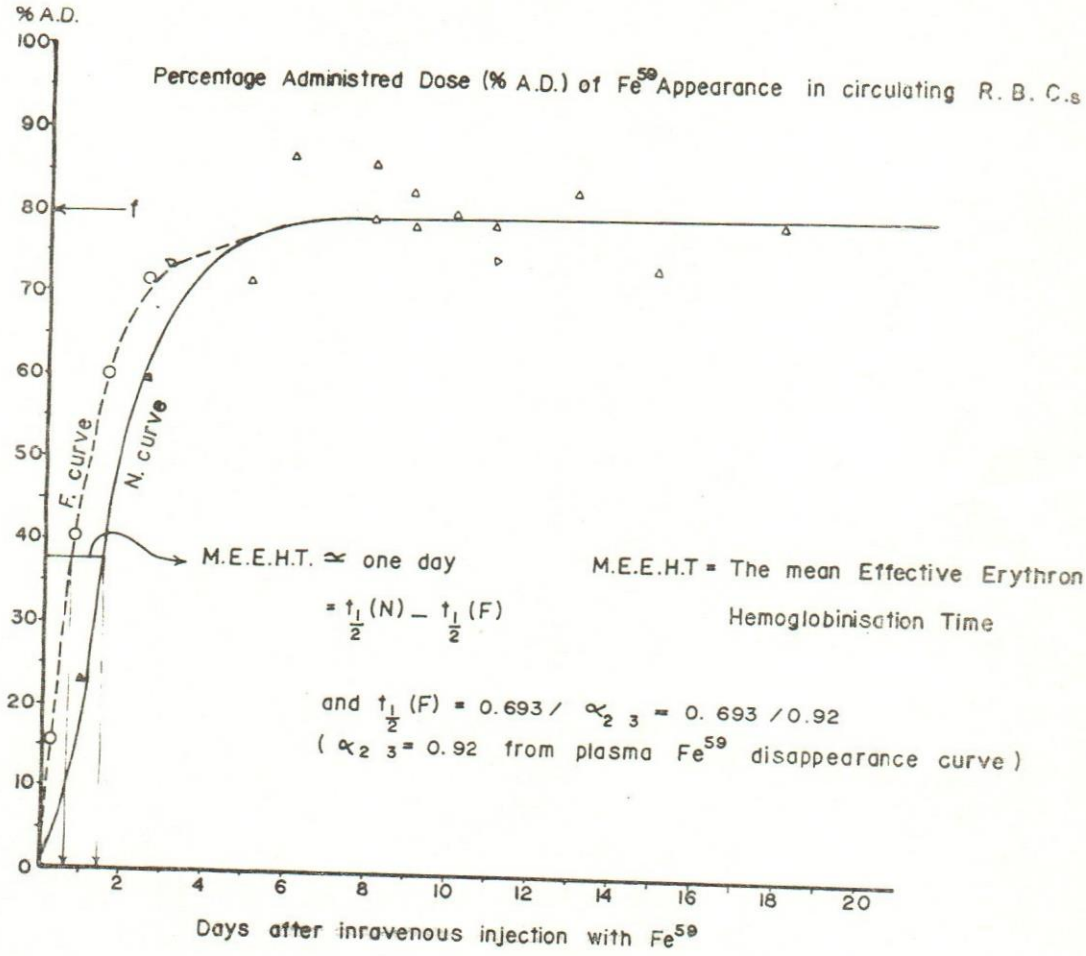

% A.D.
100
90
80
70
60
50
40
30
20
10
0
Percentage Administred Dose (% A.D.) of Fe^{59} Appearance in circulating R. B. C.s
f
F. curve
N. curve
M.E.E.H.T. ∝ one day
$= t_{\frac{1}{2}}(N) - t_{\frac{1}{2}}(F)$
M.E.E.H.T = The mean Effective Erythron Hemoglobinisation Time
and $t_{\frac{1}{2}}(F) = 0.693 / \alpha_{2\ 3} = 0.693 / 0.92$
($\alpha_{2\ 3} = 0.92$ from plasma Fe^{59} disappearance curve)
2
4
6
8
10
12
14
16
18
20
Days after inravenous injection with Fe^{59}

Fig. 2

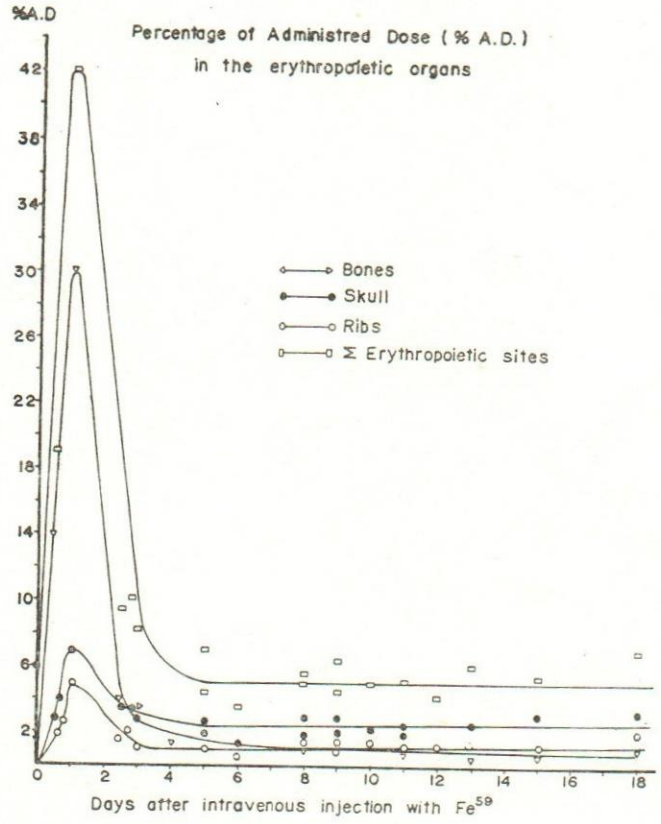

%A.D
Percentage of Administred Dose (% A.D.)
in the erythropoietic organs
42
38
34
30
26
22
18
14
10
6
2
0
2
4
6
8
10
12
14
16
18
Bones
Skull
Ribs
Σ Erythropoietic sites
Days after intravenous injection with Fe^{59}

Fig. 3

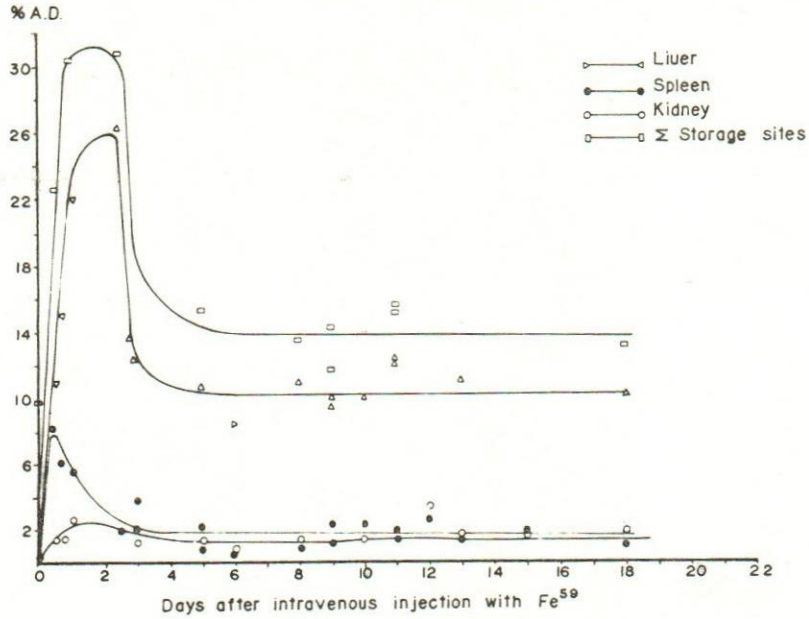
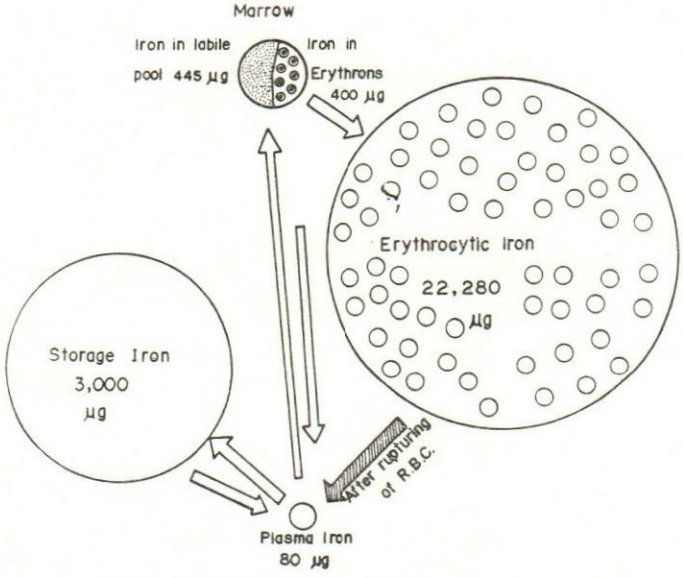


Fig. 4



Normal Distribution of Iron in Various Compartments.

Fig. 5