LXXXIII. THE RELATIONSHIP OF IRON WITH THE AGEING OF CELLS.

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THE life of the multicellular organism is divided into certain phases by developmental events which occur during those periods. During embryonic and early post-embryonic existence cell division and cell growth are exceptionally prominent. As cell differentiation increases, cell growth and cell division progressively diminish and finally shrink to a minimum. After the cell has reached its adult stage, it ages and finally dies. Thus growth, differentiation, ageing and death are stages in a continuous process. As these are stages in a process the phases gradually blend into one another.

The results of the following investigation show, for the first time, that a distinct alteration in the chemistry of the cell occurs during development. An examination of the iron content of epithelial organs showed that the cells of an organ in an old animal contain appreciably higher quantities of iron than do those of a young animal. Estimations of copper under similar circumstances did not show such variations.

The iron content of the liver, kidney, testis—and in some cases of the lung and muscle tissue—of rats, guinea-pigs, rabbits, dogs and cats was estimated, all tissue utilised in this investigation being freed from blood before chemical examination by the following methods. Immediately after death a cannula was inserted through the left ventricle into the aorta, and Ringer's solution at body temperature was infused under considerable pressure until the venous fluid was entirely free of blood. Perfusion was carried out in this manner for 20 to 30 minutes at least, and in order completely to exsanguinate the liver and kidney this was followed by further perfusion through the portal vein in the case of the liver, and through the abdominal aorta in the case of the kidney, after all the vessels except the renal artery had been ligatured. The completeness of exsanguination was controlled by histological examination.

The iron determinations were made according to Warburg's [1927] method by which iron may be detected in quantities as small as 5×10^{-4} mg. 50 mg. of thoroughly disintegrated tissue were placed in a platinum crucible and incinerated by heating for 10 to 12 hours at 800° in an electric furnace. 1 ml. of 0.1 N HCl was poured into the crucible containing the ash, and 0.1 to 0.2 ml. of the resultant solution was examined for iron. In all, this investigation, which was made at the Charité, Berlin, includes the examination of tissues from 125 animals. The greater number of estimations were on rats; but the study of other animals was sufficient to show that in them the results were similar to those in the rat.

The iron content of pulmonary tissue showed greater variations than that of the liver, kidney and testis. These irregularities may possibly be explained by the inhalation of iron during life. Hence the examination of lung tissue was carried out only in a relatively small number of rats. Similarly the spleen may contain an

appreciable amount of iron as a result of the destruction of erythrocytes, and therefore this organ was excluded.

Is the increase in the iron content of aged cells of special significance or is it dependent on the destruction of blood during life? The latter possibility can be excluded for the following reasons.

1. Histological examination did not show this increased iron to be due to haemosiderosis, and further the increased iron occurred regularly in organs, such as in the testis, in which haemosiderosis does not usually take place.

It might be claimed however that the histochemical methods for the demonstration of iron are too crude to demonstrate the minute amounts present in the cells, or to show variations when these are present.

2. The lower values for iron found in the tissues of young animals and the high values of the old are definite and constant. This is not only true in different animals of the same species but also in animals of various species during corresponding phases of development.

3. If the observed increase in the iron content of the tissues of old animals were due to iron deposited in tissues following blood destruction, then this increase should be continuous throughout life; such is not the case. According to our results the increase in the iron content of the cells in old adults is clearly associated with a definite developmental phase. The increase occurs within a short time and remains at the new level throughout the remainder of the animal's life. This increase of the cell-iron must therefore be of some functional significance.

A study of the iron values of the organs of the rat is illuminating. In the rat, the iron content of the kidney and testis is the same throughout the first 10 months of life (Fig. 1) and in the majority of these animals, even throughout the whole of the first year of life. In a certain proportion of animals, the iron content begins to increase between 12 and 15 months after birth but more frequently after 15 months.

Fig. 1. Rat.

Fig. 2. Guinea-pig.

— Liver. --- Kidney. ... Testis.

The values reach their maximum at $1\frac{1}{2}$ years, while only in a small proportion of cases is this higher level delayed until the second year of the animal's life. Throughout the remainder of the rat's existence (in all 3 to 4 years) the iron values remain the same. The increase of iron in the liver occurs at about the third month, and maximum values are obtained by the end of the fifth month.

Thus the change from the low level prevalent in the tissues of the young to the high values obtained in the old animals occurs in 3 to 6 months, a relatively short time. Indeed, this rapid increase occurring as it does at a definite interval indicates that this phenomenon is due to some physiological process and is not the result of such a coincidence as the deposition of haemosiderin in the organs.

A further point is also of importance. The rise in iron values occurred in all the types of animals examined and at the same period, that is the middle period of the animal's life.

Since the duration of life of guinea-pigs and rabbits is longer than that of rats, the increase of tissue-iron does not begin before the end of the second year (guinea-pig) or even later (rabbit) (Fig. 2 and Table I). In the liver the increase

Table I. *Rabbit*.

Iron contents of organs at various periods of life in mg. per kg.
dried substance—average values.

Age	Days				Months		Years			
	1	3	28	33	2½	3	1	1½	2	6
Liver	1400	—	—	180	160	—	240	200	200	700
Kidney	500	500	—	150	160	—	160	170	—	350
Testis	—	—	125	—	—	—	120	—	125	260

generally begins somewhat earlier than in the other epithelial organs, but the difference is not as great as in the rat. In dogs and cats of 4 to 5 years of age the low values found in the young are still present, and a definite increase in liver-iron does not occur until the seventh year. In the young animals, the results are closely grouped together (from 100 to 180 mg. per kg. of dried tissues, Tables II and III). As already noted, this is true in animals of various species (Table IV). The maximum values of the old animals show somewhat greater variation, but for the different species the readings fall into a definite group (200 to 500 mg.). In the liver the maximum values may be higher—up to 800 mg. (Tables II, III, IV B).

Table II. *Rats*.

Iron content of kidney of rats (mg. per kg. dried substance).

No. of animal	Under ½ year (young animals)	No. of animal	Over 1½ years (old animals)
1	170	1	268
2	182	2	200
3	162	3	215
4	90	4	435
5	120	5	476
6	107	6	500
7	115	7	326
8	184	8	300
9	170	9	300
10	148		
11	180		
12	123		
13	115		
14	120		
15	140		
Average	142		335

Table III. *Rat.*

Iron content of testis of rats (mg. per kg. dried substance).

No. of animal	Under $\frac{1}{2}$ year (young animals)	No. of animal	Over $1\frac{1}{2}$ years (old animals)
1	96	1	205
2	115	2	355
3	103	3	330
4	85	4	218
5	135	5	294
6	142	6	272
7	157	7	250
8	135	8	264
9	110	9	526
10	128	10	280
11	180	11	182
12	145	12	260
		13	210
		14	400
		15	335
		16	276
		17	270
		18	410
		19	180
		20	345
		21	200
		22	210
		23	270
Average	130		283

Table IV.

Average iron content of organs of animals (mg. per kg. dried substance).

A. Young animals

Animal	Liver	Kidney	Testis
Rat	180	140	130
Rabbit	170	155	125
Guinea-pig	145	140	125
Cat	200	125	—
Dog	200	150	115

B. Old animals

Rat	About 700	335	280
Rabbit	Up to 700	400	260
Guinea-pig	450	350	280
Cat	Up to 800	—	—
Dog	Up to 600	—	—

As to the iron content of the organs of young animals, Bunge [1898] found that certain animals, *e.g.* rabbits, are born with a relatively large store of iron. But according to the results of this investigation the tissue-iron of rabbits rapidly decreases in amount during the first weeks of post-embryonic life to the same low level which young animals of other species already have from birth (Table I).

Reports have already been published of the estimation of iron in the organs of animals. Very often differences have been found to exist between different species. For instance, the amounts obtained in mice and rats were much larger than those in guinea-pigs and rabbits. The probable explanation is that for small animals, such as mice and rats, the older ones have been utilised in order to obtain sufficient material, while in the case of larger animals, such as guinea-pigs and rabbits, even the organs of the very young are sufficient for analysis.

The iron values are independent of the food intake of the animals. This has been proved by depriving rats of vitamin B-containing food. In spite of this and the consequent considerable loss of weight, the iron content was always found to correspond with that of other animals at the same period of life.

The iron values in the organs of the young are so constant and the increase during age so regular that it is possible to determine the age group of an animal by iron estimations, preferably of the kidney or testis. Low iron values in the testis of rats—below 160 mg. per g. of dried tissue—definitely indicate that the animal is under $1\frac{1}{2}$ years of age, while quantities above 180 mg. suggest that the animal's age is over 10 months and most likely over 15 months. The intermediate period between 10 and 15 months cannot be determined with certainty because it constitutes the transition from the period of low iron values to that of the high values.

The iron content of the striped muscle of young animals is about the same as that found in epithelial organs, especially in the kidney and testis. From this fact we have to conclude that the iron is present not only in cells but also in the paraplasmatic substances in which muscle tissue is very rich. The rise of the iron content in old animals only occurs in the epithelial organs, not in the striped muscle. Therefore the increase of the iron content is probably due to a new iron compound.

SUMMARY.

1. The iron content of the epithelial organs in the animals investigated in this series (rats, guinea-pigs, rabbits, cats and dogs) is constant.

2. A similar constant iron value is obtained in the various organs of the same animal, in the organs of animals of the same species, and as between the organs of animals of the various species. Only in the liver are the variations appreciable, and even here these variations are relatively small.

3. At a definite period of life and within a short time, the iron content of epithelial organs is increased up to 200 %. The same iron value is then maintained until death.

4. This newly acquired iron is probably a compound other than those already present in the cell and must be associated with the ageing of the cell. Hence the longer the life period of the animal the later does the increase occur. The middle period of life can be considered the period during which the increase in all iron occurs.

5. The iron content of the organs of young animals is so constant and its increase at a definite period is so regular that from the iron content of the organs of an animal, especially the kidney and testis, it is possible to determine the age group of the animal from which the tissue has been taken. The cells of epithelial organs having a high iron content can be considered as in the decline of life.

6. Since the increase in the iron content of organs is so regular a feature, the phenomenon must have some biological significance.

REFERENCES.

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