CCXXIX. IRON, COPPER AND MANGANESE IN HUMAN ORGANS AT VARIOUS AGES

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THE usual statement in textbooks of physiology that the adult organism contains much less Fe than the young one is in noteworthy contradiction to a number of findings, some of them quite old. Lapique & Guillemonat [1889] and Lapique [1897] observed a characteristic life curve of the Fe content of blood-free human and animal livers and found higher values in the adult than in the child. The first part of this curve, however, shows a rapid drop from the high values of the newborn to the low level present in childhood. Physiologists seem to have concentrated their attention chiefly on this phenomenon and have thus often overlooked the later rise. More recently Lapique's results have been corroborated, in part at least, e.g. by Ramage *et al.* [1933] in the human, and by Lintzel & Radeff [1931] in several species of animals. One of us [Zondek & Karp, 1934] showed that in certain animals the Fe content of the organs rose considerably during a definite period of life. Since this increase occurred later in those animals whose span of life is longer, one could assume that there might exist a connexion between the ageing process of the cell and its Fe content.

In the present investigation we have extended our work to man: the literature contains little about the Fe content of human tissues and the figures found must sometimes be considered critically because of the methods used. Since, moreover, only the liver had been previously studied, we carried out a reinvestigation which included also the kidney.

In a number of cases Cu and Mn were also determined in order to show how far the life curve is typical for Fe and perhaps to contribute further information on the significance of the congenital Cu deposit.

EXPERIMENTAL

Non-haemin-Fe. An organ, e.g. liver, contains: (1) blood-Fe, of which 95–98 % is in the form of haemin-Fe (or more correctly: haem-Fe), accepting Barkan & Schales' [1937] hypothesis that there exists a small fraction of pseudo-haemoglobins; (2) tissue-Fe, of which 80–90 % occurs in the form of non-haemin-Fe whose chemical nature has not yet been defined, 10–20 % being haemin compounds such as cytochrome, catalase, respiratory enzyme etc. Although direct determinations for the latter group have been made only on the rat [Yabusoe, 1925], we may assume a similar distribution in human organs. Careful washing or perfusion of an organ can at best remove only the total blood-Fe. The residue thus contains not only non-haemin-Fe, as is usually believed, but also a certain amount of haemin-Fe, the cell catalysts mentioned above.

Here we were interested chiefly in the amount of non-haemin-Fe. A report of our experience with the various methods will be left to another paper; we

merely mention that we modified the original method of Tompsett [1935] in that we extracted the organs for a much longer time, since by the original method, only part of the non-haemin-Fe goes into solution. The same observation has been made in the meantime independently by Tingey [1937]. The method is as follows: the tissue is washed only slightly and minced finely with chrome-plated scissors; 1-2 g. of this substance are ground up in a mortar with glass powder; several ml. saturated $\text{Na}_4\text{P}_2\text{O}_7$ and 20% trichloroacetic acid are added, the mixture is further ground up and allowed to stand for 24-48 hr. or, if the tissue contains very much Fe, for 3 days. The mixture is then transferred into a wide centrifuge tube with the aid of 10% trichloroacetic acid and centrifuged; the residue is washed twice with pyrophosphate and trichloroacetic acid and the washings added to the first filtrate. Usually 5-10 ml. pyrophosphate and 10-20 ml. trichloroacetic acid were used.

Fe determinations were carried out on aliquot parts of the filtrate by the thiolacetic acid method [Hanzal, 1933; Burmester, 1934], and for control occasionally by the *o*-phenanthroline method (reducing agent: $\text{Na}_2\text{S}_2\text{O}_4$) or by the thiocyanate method (after ashing aliquot parts with H_2SO_4 and HClO_4 in order to convert the pyrophosphates into orthophosphates). All three methods gave the same results. The colorimetric estimations were performed with the very sensitive Leitz photometer, with filter 495 $\text{m}\mu$ for all three methods.

We felt it necessary to check our method of extraction with other methods, an obvious omission from previous work. The control methods were:

(1) Starkenstein & Weden's [1928] method, consisting in extraction of the non-haemin-Fe with 5 *N* HCl at 100°.

(2) Yabusoe's [1925] indirect method: the haemin-Fe is extracted with acidified methanol, the total Fe is determined in a second sample, and the non-haemin-Fe is calculated from the difference. It was found, however, that the direct colorimetric comparison of the haemin extracts, according to Yabusoe, with a standard haemin solution gave unreliable results, so that we ashed the extracts instead and determined their Fe content by the thiocyanate method of Kennedy [1927]. Table I shows the good agreement of the three methods and proves that all the non-haemin-Fe is actually extracted by pyrophosphate.

Table I. *Determination of non-haemin Fe with various methods*

(mg. per kg. dried tissue)

No.	Tissue	Tompsett method (modified)	Starkenstein method	Yabusoe (modified)
34	Kidney	175	—	163
35	Liver	—	1050	1140
61	Kidney	146	—	142
103	Liver	605	630	582
106	Liver	2400	—	2480
117	Kidney	230	—	257
143	Kidney	218	250	227

Our method, if applied to blood, extracts 2-3% of the total Fe, corresponding to the findings of Tompsett [1934] and Shorland & Wall [1936]. Since in the organs examined, from which in no case had the blood been washed out, only about 30-60% of the total Fe was in the form of blood Fe, the values for non-haemin-Fe can hardly be seriously influenced by that small fraction of "easily split-off" blood Fe (Barkan).

Total Fe. After determining the water content, dried samples of the tissue were ashed ($\text{H}_2\text{SO}_4 + \text{HClO}_4$) and the content of the ash solution determined by the thiocyanate method [Kennedy, 1927], occasionally also by the *o*-phenanthroline method [Heilmeyer & Plötner, 1937].

Copper. Aliquot parts of the ash solution were analysed by the usual sodium diethyldithiocarbamate method [McFarlane, 1932; Steussig, 1938]. We wish to mention that we were not always able to confirm the findings of McFarlane [1934] and Tompsett [1935] that the total Cu content of tissues is present in the trichloroacetic acid extract.

Manganese. With Mn, on the other hand, we found that treatment of the tissue for 24 hr. with 10% trichloroacetic acid caused complete extraction of the metal (Table II). Thus we were able to simplify the procedure considerably

Table II. *Mn content of whole liver and CCl_3COOH extract*

(mg. per kg. dried tissue)

No.	Total Mn	Mn in CCl_3COOH extract
35	6.5	6.1
81	10.0	9.2
108	8.2	8.7

by ashing merely the trichloroacetic acid extracts ($\text{H}_2\text{SO}_4 + \text{HClO}_4$) instead of the usual ashing of 5–10 g. whole tissue, which takes much time. The ashings were then transferred into a porcelain crucible, evaporated almost to dryness (sand bath), the residue dissolved in a few ml. hot 10% H_2SO_4 and 5 drops $N/10$ AgNO_3 added. After centrifuging the Mn content of the filtrate was determined colorimetrically in the usual manner after oxidation to HMnO_4 with 1 ml. saturated $\text{K}_2\text{S}_2\text{O}_8$, using KMnO_4 as the standard solution. 1 μg . Mn in 3 ml. volume gave a sufficiently deep colour for colorimetric comparison. The optimal pH conditions have recently been carefully studied by Ray [1938] so that details need not be given here.

We need not emphasize that all the usual precautions were taken to prevent contamination and also in preparing standard solutions. The amount of the gravimetrically determined Fe standard was controlled also by titration. Blanks were run in every case. Recoveries were carried out for all methods and proved to be satisfactory.

Case reports. We used the current autopsy material of the hospital during the period extending from January 1938 to April 1939.¹ In almost every case the material was from immigrants of European origin, who had lived in Palestine for the most part from 1 to 10 years. Owing to the disturbances in this country, many of our cases included perfectly healthy individuals who had been killed by shooting.

In most cases the organs were analysed within 24 hr. after autopsy. We were able, however, to confirm the observations of Borgen & Elvehjem [1937] and Okamoto [1937], that storage at 4° up to as long as 3 days had no influence on the results.

RESULTS

In Table III the values for total Fe, non-haemin-Fe, and Cu in the livers and kidneys of a series of 75 cases are listed according to the age of the individuals.

¹ We are indebted to Dr H. Karplus for supplying us with the autopsy material.

Table III. *Fe and Cu content of liver and kidney*
(mg. per kg. dried tissue)

No.	Age	Sex	Diagnosis	Liver				Kidney				Remarks
				% water	Total Fe	Non- haemin Fe	Cu	% water	Total Fe	Non- haemin Fe	Cu	
116/17	Foetus (9½ months)	M.	Still-birth (asphyxia)	76.8	2750	2300	182	83.2	962	257	—	—
125/6	New-born	M.	Still-birth	79.7	3060	2900	280	84.7	339	175	—	—
114/15	New-born	M.	Died shortly after birth. Hyperaemia of brain	79.5	—	1550	136	84.2	538	168	32	—
119/20	New-born	M.	Died shortly after birth. Asphyxia	78.3	1340	1260	80	84.0	485	—	—	—
42/3	New-born	F.	Died shortly after birth. Asphyxia	82.0	590	490	382	83.9	441	—	14	—
40	2 days	F.	Microcephalus	—	—	—	—	83.5	370	220	14	—
49/50	3 "	F.	Pneumonia. Subdural haemorrhage	80.4	380	300	270	84.2	380	—	16	—
29/30	3 "	M.	Bronchopneumonia. Haemorrhage	—	2920	2500	375	—	504	—	37	—
138/9	3 "	M.	Pneumonia. Rupture of tentorium	74.0	—	1500	—	82.8	—	250	—	—
1/2	4 "	F.	Meningitis. Spina bifida	79.4	2800	2400	332	86.4	487	—	16	—
32/33	8 "	M.	Adrenal infarct	—	1315	1000	228	—	360	—	—	Fatty liver
108/9	9 "	M.	Pneumonia	77.5	—	2020	162	84.1	—	165	—	—
A/B	13 "	M.	Enteritis. Cystitis	76.5	3200	2900	234	82.3	1160	1000	420	—
143	13 "	F.	Enteritis. Congenital heart defect	—	—	—	—	84.3	444	214	—	—
122/8	14 "	M.	Melaena neonatorum. Pneumonia	81.0	2500	2450	80	85.6	266	210	—	Transfusion given
99/100	15 "	M.	?	77.5	—	1160	240	84.0	—	190	—	—
106/7	1½ months	M.	Pylorospasm. Pulmonary oedema	78.8	2720	2420	252	85.4	—	235	—	Fatty degener. of liver
90/1	3 "	M.	Ulcerative colitis	63.7	1000	700	22	82.4	—	134	—	—
110/11	4 "	M.	Enteritis. Nephritis. Anaemia. Haemorrhage	80.6	1050	1000	76	82.4	—	158	—	—
95	4 "	F.	Meningitis. Pyuria	—	—	—	—	85.4	—	104	—	—
132	5½ "	M.	Pneumonia	—	—	—	13	—	—	—	—	—
150/1	8 "	M.	Empyem. Abscess of lungs	77.0	—	250	—	83.0	—	85	—	—
101/2	12 "	F.	Enteritis. Anaemia	75.0	1250	800	12	83.5	—	98	—	Transfusions given
17	13 "	M.	Thrombosis of portal vein	—	—	—	—	84.0	175	—	63	—
133	15 "	F.	Empyem. Pericarditis	—	—	—	—	84.2	—	120	—	—
36/7	18 "	M.	Lymphat. Leukemia. Anaemia. Tumour spleen	74.2	240	140	30	81.3	150	60	18	—
67/8	20 "	F.	Pneumonia. Cretinism	73.5	690	450	36	79.4	—	70	—	—
92	21 "	M.	Sepsis	—	—	—	—	81.8	—	57	—	—
69/70	2 years	M.	Burn	75.6	250	63	—	81.2	—	53	22	Fatty degenerat. of liver, kidney
112/13	2 "	M.	Encephalitis	75.0	—	113	—	82.0	—	94	—	—
130/1	2½ "	F.	Heart block	77.5	—	90	—	83.4	—	90	—	—
83/4	4 "	F.	Fibr. Bronchitis. Glom. Nephritis	75.1	700	200	—	79.6	—	88	—	—

Iron. In spite of the fact that many of the cases were pathological and in spite of the great variability of the Fe content even in normal individuals, a typical distribution according to ages could be recognized. This is made even clearer by a study of the statistical evaluation of the figures for the various age groups (Table IV). The significance of this phenomenon will be discussed in detail later.

Table IV. *Non-haemin Fe in various age-groups*

Age	(mg. per kg. dried tissue)					
	Liver			Kidney		
	No. of cases	Mean value	Range	No. of cases	Mean value	Range
0-15 days	14	1770	300-2900	9*	205	165-257
3-12 months	4	690	250-1000	5	116	85-158
15 months-3 years	5	170	63-450	7	78	53-120
4-12 years	5	350	200-600	5	102	88-125
16-22 "	2	705	370-1040	6	162	102-250
23-35 "	6	1040	540-2000	8	175	110-230
36-75 "	12	930	186-2500	10	160	84-256

* No. B not included.

Hitherto there were hardly any Fe values for perfectly healthy adults to be found in the literature, although Ramage *et al.* [1933] emphasized the importance of such figures some time ago. We present here the values for a series of selected cases without anatomical pathological findings, most of whom had been killed by shooting:

Liver. Total Fe (cases 103, 22, 96, 98, 6): average 1080 mg./kg. dried substance (range 700-1570).

Non-haemin-Fe (cases 144, 103, 22, 140, 96, 98, 145, 6): average 800 mg./kg. dried substance (range 550-1240).

Kidney. Total Fe (cases 62, 64, 65, 104, 23, 73, 61, 97, 34, 6): average 411 mg./kg. dried substance (range 292-540).

Non-haemin-Fe (cases 62, 64, 65, 71, 104, 58, 73, 61, 97, 34, 66, 7): average 161 mg./kg. dried substance (range 110-190).

For non-haemin-Fe, the ratio liver : kidney is 5 : 1. It is noteworthy that in various animal species different figures have been reported, e.g. in the rat 2 : 1.

The content of haemin-Fe, which is in the main dependent on the amount of blood present in the tissue examined, is about the same in liver and kidney, averaging 250-280 mg./kg. dried substance.

If one compares the average for non-haemin-Fe in the healthy individuals with the total average for all the cases (adults), there is practically no difference in the kidney. In the case of the liver, however, the total average is 20 % higher than the value found in healthy individuals, obviously owing to the fact that in a series of diseases there is a tendency for the Fe content of the liver to increase. (We shall refer later to the effect of blood transfusions.)

We were not able to observe definite differences due to sex either in the liver or kidney, in contradiction to the findings of Lapique & Guillemonat [1889]. For comparison with our absolute values, we quote the figures obtained by other authors, all in mg./kg. dried substance:¹ Lapique & Guillemonat [1889]: 1300 (new-born infants), 240 (1-2 years), 940 (male adults)—total Fe in blood-free liver; Hueck [1912]: 500 (adults, blood-free liver); Adler & Adler [1931]: 1330 (new-born infants), 447 (6 adults)—blood-free liver; Gladstone [1932]: 900 (9 newborns and infants)—non-haemoglobin-Fe in blood-containing liver;

¹ For calculation, the water contents of liver and kidney were taken as 75 and 80%.

Toverud [1935]: 1800 (47 new-born infants)—blood-free liver. That Tompsett's [1935] values are lower because of the method used has already been mentioned. He reports 270 (12 adult livers) and 35 (12 adult kidneys). For total Fe in liver we quote Ramage *et al.* [1933]: 2250 (6 new-born infants), 800 (15 children between 6–12 years).

Copper. The existence of a congenital Cu deposit in the human liver is well known and has been confirmed by this investigation also. The average value for liver in 14 infants (0–1½ months) was 230 mg./kg. dried substance, ranging from 80 to 382. After the second month the values drop rapidly to the adult level, remaining more or less constant for the remainder of life.

Our average value for adults is 34.6 (12 cases, range 16–55). Most other investigators give similar figures, e.g. Ramage *et al.* [1933] 265 (24 new-born and infants up to 7 weeks, range 50–500), 60 (24 children from 3 to 12 years); Kleinmann & Klinke [1930]: 27.5 (adults); Herkel [1930]: 25.4 (10 adults); Tompsett [1935]: 22.0 (12 adults).

In one case (No. 35) with the diagnosis of tuberculosis, we found a definitely increased value. We should not consider this observation significant, were it not for the fact that similar findings were observed in the guinea-pig [Cherbuliez & Ansbacher, 1929]. We have further studied the figures compiled by Ramage *et al.* [1933] and found that 8 of a total of 12 cases with the diagnosis tuberculous meningitis showed a definite increase over the normal values of the corresponding ages. The significance of this phenomenon seems at present obscure.

There is no congenital Cu deposit in the kidney. The average for all ages is 26.3 (range 10–70). In comparison with Fe the individual variations are much greater, which may be due to the fact that the kidney is the excretory organ for Cu, but not for Fe. Case B (cystitis, enteritis) is unusual because of the extremely high value of 420. In the same case the Fe content of the kidney was increased fivefold so that there seems to have been a serious disturbance of the metal metabolism.

As Table V shows there is no congenital Cu deposit in the brain either, in contradiction to statements found at times in textbooks of biochemistry. Since there are considerable differences in the content of heavy metals in the various

Table V. *Cu content of brain*
(mg. per kg. dried tissue)

No.	Age	Cu
121	New-born	15
46	New-born	20
124	14 days	6
16	13 months	14
9	16 years	50
56	17 "	23
80	36 "	35
5	75 "	32

parts of the brain, a more detailed study of this organ can be made only after careful anatomical dissection. Recently Tingey [1937; 1938] made an excellent study of the subject, and since his methods are almost identical with ours we can well compare his values with our figures for the remaining organs.

In addition, we made a few analyses, intended to be merely of an exploratory character, of several endocrine glands. We found surprisingly low Cu values for thymus, hypophysis and thyroid, between 4 and 20 mg./kg. dried substance. The Fe content of the same organs was between 100 and 300 mg./kg. dried substance.

Manganese. Table VI shows the Mn content of several livers. There seems to be no influence of age. The values are fairly constant, ranging between 4.5 and 10 mg./kg. dried substance, with the mean value of 7.0, identical with that of Richards [1930] and similar to the value 8.4 of Ramage *et al.* [1933] for 24 cases ranging in age from 3 to 12 years.

Table VI. *Mn content of liver*

(mg. per kg. dried tissue)		
No.	Age	Mn
42	New-born	4.8
49	3 days	6.0
108	9 "	8.2
99	15 "	10.0
36	18 months	10.0
67	20 "	9.1
69	2 years	4.8
83	4 "	7.4
81	7 "	10.0
38	26 "	4.5
35	38 "	6.5
96	44 "	5.4
52	70 "	5.2

Several exploratory determinations for serum, for which 50–100 ml. serum had to be used, gave values from 1 to 3 μg per 100 ml., in accordance with Bertrand & Medigreceanu [1912] and in contradiction to the higher values of Reimann & Minot [1920].

DISCUSSION

Iron

As may be concluded from Tables III and IV, the typical life curve for total Fe and particularly for non-haemin-Fe is present not only in the liver, as was observed by Lapicque & Guillemonat [1889] and Ramage *et al.* [1933], but also in the kidney. In the latter organ the regularity is even more distinct, since the individual variations are fewer. What is the significance of this curve?

The values in the newborn are in general quite high, but there are exceptions. According to the classical theory [Bunge, 1898; Abderhalden, 1900] the newborn has a congenital reserve deposit of non-haemin-Fe, which is to compensate for the lack of Fe in the milk diet of the first few months. But a series of investigations indicate that this is true only in part [Schwartz *et al.* 1924; Adler & Adler, 1931; Gladstone, 1932; Ramage *et al.* 1933]. Some increase in non-haemin Fe occurs already during foetal life, but the bulk of it is not accumulated until after birth, when the well known post-natal blood destruction reduces the high haemoglobin values of the newborn to about 50 % within a few weeks, liberating large amounts of Fe.¹ This "mobilization of Fe metabolism" [Schwartz *et al.* 1924] is obviously dependent on the circumstances of birth, since the livers of infants born by Caesarian section and still-births, according to the authors quoted above, usually show low Fe values.

An interesting parallel to this are the recent observations of Horváth & Hollósi [1935] that the appearance of the polycythaemia neonatorum is also dependent on the circumstances of birth. Infants born by Caesarian section do not show an increased red cell count. Sachs *et al.* [1938], on the basis of similar findings, have suggested that the shock produced by the trauma of birth might

¹ Part of the liberated Fe is excreted, resulting in a negative Fe balance during the first 2 months [Stearns & McKinley, 1937].

lead to an increase in the erythrocytes. Obviously in the newborn large blood reserves can be put into circulation quite suddenly. Applied to our problem we must assume the following mechanism: birth $\rightarrow$ shock $\rightarrow$ polycythaemia $\rightarrow$ blood destruction $\rightarrow$ accumulation of the liberated Fe in the organs. Abnormal low Fe values, as in our cases Nos. 42 and 49, could thus be explained not only by Fe deficiency of the mother, but also on the basis of an absence of the shock reaction.

On the other hand one would expect low Fe values in every still-born infant. Yet our two cases (Nos. 116 and 125) concerned have unusually high values. Either the mechanism described is not correct, or it can occur in the uterus before birth, perhaps as the result of insufficient O₂ supply to the foetus. We intend to study this question further in the future.

The further course of the curve shows a definite decrease in the Fe values to a minimum which is reached at about 2 years. According to the classical theory mentioned above, the Fe reserves are called upon during lactation for the formation of blood and muscle haemoglobin, since mother's milk with its very low Fe content cannot satisfy the demands of the growing organism. We would expect, then, to find the lowest values after weaning, not at the age of 2 years, after the child has lived on a normal diet (rich in Fe) for some time. Ramage *et al.* [1933], who made the same observation, have previously called attention to this discrepancy.

It is similarly difficult to interpret the next part of the curve, a slow increase, more rapid after the 12th year of age, which reaches the final level of the adult between the 16th and 20th years. A similar rise in the Fe content of the organs of various animals was already reported in a previous publication [Zondek & Karp, 1934]. Since this rise appeared later in the species of animals whose span of life is longer, always in the middle period of life, it was suggested that there exists a relationship between the ageing process of the cell and its Fe content. In man the increase in the Fe values occurs much earlier, ending between the ages of 16 and 20, so that it is questionable whether it is based on the same phenomenon. But here too there is a definite period of life during which the increase occurs.

Fig. 1. Life-curves for the haemoglobin content of blood (Williamson [1916]) and the non-haemin Fe content of kidney.

The interpretation is made clearer by a comparison of our curve for non-haemin Fe with the familiar life curve for haemoglobin [Williamson, 1916] (Fig. 1). The course is so similar that we must assume a connexion. (The curve

for serum-Fe runs parallel in general, to judge by the few figures available for this Fe fraction during childhood [Thoenes & Aschaffenburg, 1934].)

We think it improbable that the so-called "physiological anaemia" of childhood can be explained by exogenous Fe deficiency. The diet of the normally fed child after weaning theoretically includes enough Fe to cover even the increased requirement of the growing organism. One might assume that the absorption of Fe is poorer than in the adult, but Hahn *et al.* [1938] were recently able to demonstrate in dogs by means of the radioactive indicator method ("labelled" Fe), that, particularly in Fe-deficient anaemias, the absorption of Fe, normally very slight, is greatly improved. The good effect of Fe therapy, so well known in such cases, need scarcely be mentioned. Further, if there were an extrinsic lack of Fe we might expect that the shape of the curve for non-haemin Fe, which chiefly represents the stored reserve Fe, would be a different one. In that case all the Fe reserves would be steadily used up for the formation of new haemoglobin, and the increase would not begin until after the cessation of growth, not at the end of the 2nd year as it actually does.

All of this points against Fe deficiency. If one does not wish to accept any other deficiency or a defect in the haematopoietic system during childhood, one is forced to the opinion that the young growing organism obviously requires less haemoglobin, and therefore less reserve Fe than the adult. During a certain period of development the requirement in both Fe fractions increases, a phenomenon which is reflected in the course of the two corresponding curves.

In the discussion of the adult values we do not wish to delve too much into the clinical details, but rather to confine ourselves to the following points. In the kidney we were unable to observe any differences due to sex. In so far as the liver is concerned, the three female cases were well under the average, but in so small a series it is impossible to draw any conclusions. We must therefore assume that the definite differences due to sex which Lapicque & Guillemonat [1889] observed, were more conspicuous in his larger series.

The Fe content of the kidney is strikingly constant. Pathological influences are of hardly any consequence here, and in anaemias the Fe reserves of the liver seem to become exhausted much sooner than those of the kidney (case no. 26/27).

It was very interesting for us to observe how low the Fe content of the organs could be reduced as the result of severe anaemia or from other causes, considering the view of Hahn & Whipple [1936; 1938] that it is impossible to go below a certain minimum concentration. In the dog this "parenchyma-Fe" fraction which seems to be essential to life, is about 60 mg./kg. dried substance, of the same order as our minimum values in cases nos. 36/7, 69/70, 112/13, 130/1, 148/9. The last case demonstrates also that all the Fe acquired during growth can serve as reserve Fe in case of a severe anaemia.

Among the few factors which can definitely influence the Fe content we must above all mention blood transfusion. Gladstone [1932] showed in a detailed study that the destruction of the foreign blood which occurs in the reticulo-endothelial system promptly increases the Fe content of the spleen and later of the liver. Lapicque [1897] made the same observation in animal experiments. As our cases nos. 101, 60, 85/6 show, we are in complete accord with these authors and can also include the kidney among the organs which are able to take up the Fe liberated by the destruction of the foreign red cells.

Copper

The catalytic influence of Cu on haemoglobin formation has now been conclusively proved for quite a series of animals [Elvehjem, 1935; Potter *et al.* 1938] and it seems probable that man belongs to this series. Cu influences the mobilization of the Fe deposits and their transformation into haemoglobin. It is generally believed that the congenital Cu deposit bears some relationship to this function. So far two hypotheses have been built up on this assumption: (1) Cu acts locally at the site of blood formation, which in the foetus is chiefly the liver; (2) Cu is stored in the foetal liver to compensate during the lactation period for the low Cu content of the milk. The shape of the Cu curve with its sudden decline after the second month is an argument against the latter point of view, because a more gradual decline during lactation should otherwise occur; but there are many possible objections to the first hypothesis as well. A comparative investigation of many animal species with regard to their congenital Cu and Fe deposits, foetal blood formation and the haemoglobin and Cu contents of the blood at birth would help to elucidate this question. So far preliminary studies of this kind have been made only by Cunningham [1931] and McFarlane & Milne [1934]. A further step toward the comprehension of the action of Cu has recently been made by the isolation of a Cu compound from liver and blood [Mann & Keilin, 1938].

Manganese

Whereas Mn seems to have an influence [Kemmerer *et al.* 1931; Daniels & Everson 1935] on the growth and sexual function of rats and mice, no function at all has been observed so far in man. But the Mn content of the liver (and of other organs) is so constant that it can hardly be considered an accidentally occurring element. The important discovery of Edlbacher & Pinösch [1937] and Edlbacher & Baur [1938] that almost all the Mn of the liver (cat, cattle) is found in the arginase extract of this organ and there represents the prosthetic group of that enzyme, points to a relationship between the Mn content and protein metabolism which it would be worth while to investigate further.

SUMMARY

1. After improving on certain methods, determinations were made of the content of total Fe, non-haemin-Fe, Cu and Mn of liver and kidney tissues at various ages. Occasionally some other organs were also examined.

2. In the case of liver and kidney, a characteristic life curve exists for the % of total Fe and particularly of non-haemin-Fe. Values are high at birth, then drop to a minimum which is reached at the age of 2 years, to rise again at first slowly, later more rapidly. From the age of 20 till the end of life, the values remain at the same level.

3. In the healthy adult the non-haemin-Fe contents of the liver and kidney are remarkably constant. The mean values are 800 and 160 mg./kg. dried substance respectively.

4. Severe anaemias may reduce the Fe content of the organs to a minimum which is identical with the minimal values of childhood and within the same limits as the "parenchyma-Fe" fraction found in the dog by Hahn & Whipple.

5. Blood transfusion is one of the few factors which definitely increase the non-haemin-Fe of both organs.

6. The life curve of the liver-Cu content shows the familiar maximum at birth, which is followed by a sharp decline after the second month. The mean

value for adults is 34.6 mg./kg. dried substance. The kidney and brain do not show maximum values at birth. The Cu content of several endocrine glands is surprisingly low. In a case of tuberculosis the Cu content of the liver was markedly increased, a phenomenon which seems to be of significance.

7. The Mn content of the liver is constant during the whole of life, 7.0 mg./kg. dried substance. The content of several sera is 1–3 μ g. per 100 ml.

8. The results are discussed in detail in the light of our present knowledge of the subject.

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