REMARKS

ON

THE ANALYSIS OF IRON IN THE LIVER AND SPLEEN IN VARIOUS DISEASES AFFECTING THE BLOOD.

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THE IRON OF THE LIVER AND SPLEEN IN HEALTH.

It is now generally accepted that under healthy conditions the liver stores up a certain amount of iron, which is used for the ordinary physiological needs of the organism, and which further serves as a reserve of iron which can be drawn upon at once whenever there is a sudden demand for the rapid formation of new red corpuscles, such, for instance, as occurs after hæmorrhage. The proof of this is, that all healthy livers contain iron (apart from what is found in the hæmoglobin of the blood), and further, that after hæmorrhage, in man or animals, or after feeding animals on a diet poor in iron, the quantity usually present in the liver is found to be very greatly reduced in amount. Besides this, when an inorganic iron salt is injected into the blood, fully one-half of it is rapidly taken up by the liver cells. In the same way the spleen cells always contain iron.

The necessity for such a reserve store is due to the small amount of iron in an ordinary dietary. I have shown¹ that the amount ingested daily in the food amounts only to about 6 to 11 milligrammes (say $\frac{1}{10}$ to $\frac{1}{8}$ gr.), and the daily loss from the body is approximately about the same. Hence such a reserve in the liver maintains the iron balance of the blood, and enables it to make good very readily small bleedings or other occasional demands on it. For example, healthy women do not become anæmic from menstrual loss, while those who are already anæmic, and in consequence have no such reserves of iron, suffer markedly. The iron of the liver is derived partly from the food and partly from effete red corpuscles, the colouring matter of the latter being excreted as biliary and other pigments, while the iron is retained in the body.

The nature of the iron compounds of the liver was first shown by Zaleski,² who found that the metal was present in different organic combinations, some being very much looser than others, and that they occupied both the body and nucleus of the cells. Vay³ has also found that the iron is combined with albuminous bodies in very different degrees of intimacy, and that its amount varies greatly in health and chronic illness, or during good and poor nutrition, and is diminished after hæmorrhage—results which have been arrived at by numerous previous observers.

From some of the nucleo-albumins of the liver the iron present cannot be separated by ordinary chemical reagents nor even by artificial digestion, and its presence can only be demonstrated by examining the ash left after burning. This of course means that the combination is extremely intimate. Vay found also that a strong and immediate reaction to the ordinary tests meant a large amount of loosely combined iron in the connective tissue, leucocytes, and liver cells. I have myself often observed that the characteristic colour reactions for iron develop much more slowly in some livers and spleens than in others, the inference being that in the latter we have to do with a looser albuminous combination of the metal, and hence its iron is attacked much more readily. It seems, therefore, that a portion of the iron of the liver forms an integral part of the protoplasm of the cells and their nuclei, and only changes as they change, whereas another portion is, as it were, "floating" iron, and supplies the metabolic needs of the organism. It is the latter which is affected by varying conditions of nutrition, by hæmorrhages, etc. Even in the most profound anæmia a certain

amount of iron can always be found in the liver, namely, that portion which is so intimately combined with the albumin of the cells, and which gives no colour reaction to ammonium sulphide or to ferrocyanide of potassium *plus* hydrochloric or sulphuric acid. As the iron of some nuclein compounds is not extracted by artificial digestion, it seems very likely that a certain amount of the metal in our food is never utilised by the body and hence is useless as a source of iron.

As regards the amount of iron present in the liver a most excellent critical summary of the results of previous observers has been made by Dr. William Hunter,⁴ from which it appears that in 100 g. of the dried organ the average amount is 0.078 g. Since then Vay has found it to average 0.08 to 0.1 g., and in four cases in healthy livers (see Table, I—IV) I have found it to vary from 0.07 to 0.09 g. per 100 g. of the dried organ. In my fifth case it was very much less, but death was due to chronic nephritis, a disease which is notoriously accompanied by anæmia. My analyses give the *total* amount of iron in these four healthy livers as varying from 0.182 to 0.310 g. (about $\frac{2}{10}$ to $\frac{4}{10}$ grains). Bunge⁵ reckons the total iron present in the body of a healthy adult as 2.5 g. (38 grains) so that the amount stored in the liver forms a pretty large proportion of the whole.

It is very difficult to gauge the quantity of iron present in the spleen, apart from that forming the hæmoglobin of the blood. The spleen cannot be washed free of blood as the liver can—it varies enormously in weight and in the amount of blood it contains, and hence, in making any analysis, we always get the iron proper of the spleen *plus* a variable amount of the metal derived from hæmoglobin. For this reason a determination of the amount of iron in the spleen is of relatively little value, as we cannot say how much is actually present in the spleen cells, and how much in the blood corpuscles. In nine analyses Stahel⁶ found the iron per 100 g. dried to average 0.182 g., and in four cases (see Table, I—V) I found it to vary from 0.144 to 0.4 g. The total amount in the spleen I found to vary from 0.026 to 0.294 g. (about $\frac{1}{10}$ to $\frac{4}{10}$ grains). The greater part of this is probably derived from the hæmoglobin, and my opinion is that the iron stored in the spleen is normally very much less than in the liver, to which also it bears no constant proportion.

THE IRON OF THE LIVER AND SPLEEN IN DISEASE.

I have made analyses of the amount of iron in the liver and spleen in various conditions affecting the blood, and have summarised the results in the accompanying table. A glance at the figures will show that the quantity found varies within very wide limits, but that iron is never absent entirely even after death from the profoundest anæmia. The analyses I—IV are those of healthy livers and spleens supposed to contain a normal amount of iron; VI—XIII are from cases in which the iron was more or less greatly increased; and XIV—XXI in which it was diminished.

In all the cases VI—XIII (with the exception of the case of Addison's disease) there was extreme anæmia, and there were more or less extensive internal interstitial hæmorrhages. In all of them, also, excess of iron was found in the liver, but not necessarily in the spleen. In fact, the spleen varies much in this respect, being in most cases quite comparable with the normal condition. The excess in the liver varied from about $\frac{1}{2}$ to 4 times the normal.

In the cases XIV—XXI the anæmia was also extreme and was in all the immediate cause of death, but in them the blood loss was external (either by direct bleeding or from suction by the ankylostoma worm). In these the amount of iron in the liver was always extremely small, and the organ gave no indication of containing iron when the ordinary chemical tests were applied. Probably, therefore, all the iron, except what was most intimately combined with the protoplasm of the cells, had disappeared. The diminution of iron in the liver varied roughly from about one-half to three-quarters of the normal amount. In two cases the total amount of iron in the spleen was only 3 and 5 milligrammes (about $\frac{1}{10}$ and $\frac{1}{20}$ gr.), while in the third case (ankylostomiasis) it was not much under the normal.

¹ *Journal of Physiology*, 1895.² *Zeit. f. physiol. Chemie*, x, 453, 1886.³ *Zeit. f. physiol. Chemie*, xx, 577, 1895.⁴ *Lancet*, ii, 1888.⁵ *Abhandlungen des Congress. f. innere Med.*, 1895.⁶ *Virchow's Archiv*, lxxxv., 1881.

No.	Case.	Iron in 100 g. dried.		Iron in whole Organ.	
		Liver.	Sp. een.	Liver.	Spleen.
I	Pulmonary embolus (woman, 49) ...	G. 0.070	G. 0.144	G. 0.182	G. 0.026
II	Obstruction of bowel (man, 52) ...	0.090	0.150	0.310	0.120
III	Myxœdema (woman, 60) ...	(1) 0.090	—	0.293	—
IV	Acute tuberculosis (man, 16) ...	0.070	0.400	0.228	0.166
V	Chronic nephritis (woman, 24) ...	0.035	0.210	0.100	0.294
VI	Pernicious anæmia (2) ...	0.230	0.086	0.722	0.013
VII	" (3) ...	0.140	0.170	*0.411	—
VIII	Tapeworm anæmia (4) ...	0.166	0.366	*0.488	—
IX	" (5) ...	0.262	—	*0.655	—
X	Malaria (chronic) (5) ...	0.257	0.110	*0.654	—
XI	Leucocythæmia (6) ...	0.337	0.290	2.640	0.435
XII	Addison's disease (7) ...	0.120	0.120	0.396	0.102
XIII	Hæmorrhage into pancreas, etc. (8) ...	0.160	—	0.580	—
XIV	Gastric ulcer and hæmatemesis (9) ...	0.018	0.041	0.040	0.005
XV	Anæmia with bleeding into bowel (10) ...	0.020	0.020	0.050	0.003
XVI	Anæmia from ankylostomiasis (11) ...	0.021	—	*0.053	—
XVII	" " " " (12) ...	0.020	—	*0.058	—
XVIII	" " " " " " ...	0.030	—	*0.088	—
XIX	" " " " " " ...	0.050	—	*0.147	—
XX	" " " " " " ...	—	0.094	—	—
XXI	Anæmia from bleeding uterine poly- pus (13) ...	Very little	—	—	—

* Those marked with an asterisk only give approximately the total amount of iron in the whole liver, the numbers being put in merely for the sake of comparison with the others. I had no note of the weights of the livers in these cases, and in calculating the total amount of iron from the analysis of part of the liver I took 1.470 g. and 1.270 g. as the average weight of the organ in man and woman respectively.

1. These five livers and spleens were given to me by the pathologists of the Royal Infirmary as being perfectly healthy, and in all respects normal.

2. Liver and spleen gave with ferrocyanide of potassium and hydrochloric acid, and with ammonium sulphide, very marked iron reactions. The convoluted tubules of the kidney also showed the iron reaction, and one kidney was found to contain 0.015 g. Fe. There was a thin layer of hæmorrhage under the dura mater. The basal ganglia of the brain gave a very marked iron reaction, the white matter gave it slightly, as did also the inner surface of the dura mater. The fat on the heart and elsewhere also reacted to the tests for iron. The bone marrow was red and pulpy.

3. This patient had suffered at intervals for some years from bleeding piles. There were numerous small hæmorrhages in the bowel and kidney, and on the surface of the lungs. Liver, spleen, kidneys, and fat gave marked iron reaction.

4. These specimens were very kindly sent to me by Dr. Ossian Schauman, of Helsingfors, the author of a most interesting monograph on the anæmia caused by the tapeworm, *Bothriocephalus latus*. The first was from a lad aged 18, the second from a woman aged 36, both being typical cases which ended fatally. The organs gave the iron reaction very deeply.

5. This specimen I owe to the kindness of Dr. Hayman Thornhill, Colombo, Ceylon. The patient suffered from extreme malarial cachexia.

6. This patient had extensive purpuric bleeding for six weeks before death, and there were also numerous internal hæmorrhages. The liver weighed 6 lb. 15 oz., the spleen 1 lb. 10 oz.

7. Caseous degeneration of suparenal capsules and acute tuberculosis of peritoneum were found *post-mortem*. No tubercles elsewhere. The corpuscles and hæmoglobin were found to be normal during life.

8. This patient was under the care of my friend, Dr. William Russell. He was aged 52, and during life was very anæmic—corpuscles 2,000,000 per c.mm., hæmoglobin 22 per cent. After death a large hæmorrhage was found in and around the pancreas, and there were small hæmorrhages under the skin and elsewhere. The liver and spleen gave deeply the iron reaction.

9. A girl, aged 18, had been bloodless for some months previous to the hæmatemesis. The heart was in a state of marked fatty degeneration. None of the organs gave the slightest trace of reaction to the tests for iron.

10. Lad, 17, had suffered for some time from intestinal bleeding, which continued until death. At the necropsy there was a found a large amount of jelly-like blood on the mucous coat of the intestine, apparently from oozing, as no ulceration was found. The heart was very fatty, the bone-marrow red and pulpy. The liver and spleen gave no iron reaction.

11. The next three livers were very kindly sent by Dr. Hirsch, of Rewa, Fiji. They are from Indian coolies who died of anæmia caused by the *Ankylostoma duodenale*. The livers were very fatty, and gave no trace of iron reaction.

12. These two specimens I also owe to the kindness of Dr. Thornhill, Colombo, who has written largely on the subject of ankylostomiasis. In both cases he found the parasites in large numbers in the intestinal canal.

13. Woman, aged 51, was extremely anæmic, the red corpuscles being 1,000,000 per c.mm., and the hæmoglobin barely 10 per cent. The heart was in a state of extreme fatty degeneration, the bone marrow red and pulpy. The liver and spleen gave no trace of iron reaction.

BEARING ON PERNICIOUS ANÆMIA.

The interest of these analyses depends largely on the light which they throw on the nature of pernicious anæmia. In a

former communication⁷ I have endeavoured to show that so-called "pernicious anæmia" is simply a congeries of clinical symptoms, and not a disease *sui generis*, and that the intense bloodlessness may be caused by many diseases, such as atrophy of the stomach, malignant tumours, prolonged diarrhoea, etc., or by conditions such as starvation and hardship, pregnancy, lactation, and hæmorrhage. Further, the anæmia may bring on fatty degeneration of the small vessels, and this gives rise in some cases to numerous capillary interstitial hæmorrhages and to larger losses of blood from the mucous membranes, which intensify the already existing bloodlessness. The blood-forming power of the bone marrow gets exhausted from malnutrition, or cannot keep pace with the blood losses, and hence extreme and fatal anæmia ensues. The condition is therefore due to insufficient formation of red blood corpuscles, but many of what are commonly regarded as the most typical features of "classic" pernicious anæmia are, I hold, dependent on the occurrence of these capillary hæmorrhages. I refer especially to the yellow colour of the skin, blood serum, and fat, the excess of iron in the liver and other organs or tissues, and the increase of urobilin in the urine, the yellow pigment and iron being derived from the extravasated blood. Clinically some cases show these symptoms very markedly, others very slightly, and in all they vary from time to time, the differences being due, I believe, to the varying numbers and to the temporary absence or presence of these hæmorrhages. It may be directly observed that in the retina they come and go, or may be entirely absent. In all cases, however, it is the intense bloodlessness which is the cardinal feature of the disease, and this may be consequent on many serious antecedent conditions, while the lemon tint of the skin, the deeper yellow colour of the serum and urine, which have been so much insisted on as clinical symptoms, are simply the result of changes in the extravasated blood, and are of quite secondary import. The same is true of the excessive amount of iron found *post mortem* in the liver and elsewhere.

In cases primarily consequent on such causes as atrophy of the stomach, prolonged hardships, or diarrhoea, there is in all probability exhaustion of the bone marrow from malnutrition, or a failure to make blood owing to non-supply of the necessary pabulum, while in cases due to blood loss, as in ankylostomiasis or uterine bleeding, the marrow fails to keep pace with the loss, and ultimately may become exhausted. The latter hypothesis is rendered probable by the fact that in extreme cases of ankylostomiasis recovery does not take place after expulsion of all the parasites. Where the bleeding is external there is apparently very little tendency to internal hæmorrhages, but the broad symptoms of extreme anæmia and the changes of shape in the red corpuscles are similar, and clinically they are in the end indistinguishable.

What we are accustomed to call pernicious anæmia seems to me, therefore, to be simply one mode of termination of some serious and some necessarily fatal diseases, the great feature being the excessive anæmia, all the other symptoms and changes being consequent on this.

Several observers—but especially Quincke, Hunter, and Mott—have drawn special attention to the large amount of iron in the liver and spleen as being a condition peculiar to "true" cases of pernicious anæmia, and distinguishing it from all other forms. The first named, so far as I know, has expressed no definite opinion as to its origin, but Dr. Mott holds that the iron is derived from the destruction of blood corpuscles directly by the liver cells, and Dr. Hunter that the red discs are destroyed in the portal circulation by a ptomaine formed in the intestine and afterwards absorbed. I have previously⁸ criticised these views in some detail, and pointed out that there is no sufficient proof that the liver cells destroy red corpuscles either in health or in disease. As regards Dr. Hunter's theory the proofs advanced rest partly on experiments with toluidine and other blood-destroying drugs, and partly on clinical observation. He found experimentally that the administration of certain substances to animals broke down the red corpuscles, the iron of which was absorbed by the liver, and that clinically in man there was marked gastro-intestinal

⁷ BRITISH MEDICAL JOURNAL, I, 1895⁸

⁸ BRITISH MEDICAL JOURNAL, loc. cit.

disturbance, with the presence of cadaverine and putrescine in the urine. These alkaloids are in themselves comparatively innocuous, but he argues that the same organisms which have produced them may also have produced another ptomaine capable of breaking down the red corpuscles. Such a body, however, he did not actually find. The theory is developed at length in connection with a case which he has published in detail,⁹ but where *post mortem* very complete atrophy of the gastric mucous membrane was found. It has been known for long that pernicious anæmia is associated with this condition, cases having been described by Fenwick, Brabazon, and many foreign observers, and where such a serious lesion exists it seems hardly necessary to call in the aid of a special microbe or a special poison manufactured by it, to account for the bloodlessness. In such a case as this the intestinal mal-digestion, and the unusual putrefactive processes would seem to be immediately referable to the stomach condition, on which the anæmia also probably depends.

On the other hand, a study of the accompanying analyses of the liver shows that pernicious anæmia is not the only condition in which excessive amounts of iron are found in that organ, nor is the quantity of the metal greater in it than in some other diseases. The case of Addison's disease may be disregarded, as we have no knowledge of its influence on the blood; in malaria the corpuscles are destroyed by organisms which lodge in them, and the broken-down products and iron pigment may be found all over the body, the iron being largely taken up by the liver cells. In the other cases there had been interstitial bleedings, and it is from these, I believe, that the excess of iron found in the livers had been derived.

In those cases of fatal anæmia, where the blood loss was external, very little iron was found in the organs. Further, after artificially-made subcutaneous bleedings in rabbits, and during the absorption of large internal hæmorrhages in man—hæmatocele, scurvy, etc.—iron is plentifully deposited in the liver, and a large amount of yellow pigment is formed from the hæmoglobin of the extravasated blood.

These facts seem to bear out my view that the deposit of iron in the liver and elsewhere and the general diffusion of yellow pigment are not peculiar to pernicious anæmia, but occur in consequence of the interstitial hæmorrhages which take place in some cases of extreme anæmia.

METHOD OF ANALYSIS.

In conclusion, I may shortly describe my method of analysis, as it may be of interest to those who are working at the subject. One hundred grammes of liver were cut up into small pieces, and the blood carefully washed out with water. It was put into strong alcohol for a few days, pounded in a mortar, and then dried, first on the water bath and afterwards in an air chamber at 105° C. The loss of water was determined by weighing it. Ten grammes were then gradually added to fused nitrate of potassium in a porcelain capsule kept hot by a Bunsen flame, and when the organic matter was all burned off the iron was left as ferric hydrate. The contents of the capsule were then taken up with hot water, and filtered, the ferric hydrate, which is retained on the filter, being repeatedly washed with water, and then dissolved in dilute sulphuric acid (1 to 4). As there are always reducing substances present, the solution was heated on the water bath with gradually added quantities of solution of potassium permanganate until reduction no longer took place. If an excess of the permanganate solution had been added, or if binocide of manganese had been formed, these were got rid of by adding sufficient peroxide of hydrogen solution, the excess of this being driven off by boiling for a few minutes. The solution was then merely tinged pink with permanganate of potassium, and allowed to stand for a few days, when, if no further reduction took place, the whole was reduced with zinc and titrated with a standard solution of potassium permanganate. By this method great accuracy is obtained, as in such analyses there is a tendency to get too high results owing to the presence of organic matter. The reagents and utensils used were all proved to be free from iron. The spleens were treated in the same way, but the blood could not be washed out of them.

⁹ BRITISH MEDICAL JOURNAL, ii, 1890.

REMARKS

ON

OCULAR SYMPTOMS OF GENERAL PARALYSIS OF THE INSANE, WITH SPECIAL REFERENCE TO ITS CLINICAL GROUPINGS.

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(Concluded from page 1025.)

SECOND GROUP.

This embraces the subjects just referred to, being characterised specially by the occurrence of cycloplegic iridoplegia. The cases comprised in this category are sufficiently defined in their history, symptomatology, and course as to claim the position of a separate group.

(a) *Mental Symptoms.*—These, in an early stage, are marked by a light-hearted buoyancy arising from a general feeling of *bien-être*—a peculiarly self-satisfied and nonchalant air, which is associated with much playful gaiety and a careless, flippant manner betokening early dementia. In fact, such cases may be described as childish, good-humoured, and addicted to levity and pranks. Their egoism is occasionally indicated in their criticism of others (to their obvious disadvantage) associated with them—in their argumentative tone—love for a display of their own abilities, and the tendency to ridicule others. They are usually very careful in their attire, and their conduct throughout indicates self-admiration and content. Memory is early implicated; attention is enfeebled, but early moral flaws are not so obvious as in other forms. They are talkative, jovial busy-bodies, fond of hoarding up, and almost invariably entertain exalted notions and ideas of great wealth and prosperity. Acute excitement is apt to occur usually in association with convulsive attacks which are present in a large proportion of such cases towards the end. In one notable case, acute aural and visual hallucinations were present for several months before the fatal termination.

(b) *Speech Troubles.*—Articulatory troubles, although a very notable feature in all these cases, was a later sequel to the oculo-motor. Thus in the case of J. M. well-marked loss of both iridal reflexes and accommodation accompanied a very slight impairment of articulation, and this only perceived during the enunciation of difficult test words; whilst in G. H. B. the enunciation was perfectly clear under all conditions. It is of interest to note that the thick, drunken, laboured, and hesitating utterance—the lips compressed to limit their tremulousness, and the resulting speech an indistinct muttering are the usual anomalies noted; and that where cases present disturbances of equilibration and ataxy, the speech is at times notably explosive, jerky, and forcible, so very different from the mumbling speech of the former. Both forms eventually approach utter unintelligibility.

In connection with the last-mentioned case, it is of interest to note that he presented at one time an instance of epileptiform seizures which could be induced whenever he was requested to protrude the tongue. I find on reference to my notes the following:

G. H. B., for the past six months, has been suffering from numerous convulsive seizures, and again last night he had a generalised convulsion. This morning he talks with a somewhat thick utterance but not much apparent effort, and is quite clear mentally, recognising all that is said to him, and replying concisely and reasonably. Asked to protrude the tongue he fails to do so; evidently it costs him much effort. When urged to do so he partially succeeds, but a spasm immediately affects left side of cheek, and spreads into a genuine convulsive seizure limited to the face; the mouth was drawn to the left side, and the muscles of the face on this side were thrown into a state of violent tonic twitching. No convulsion of arm or leg occurred, and during the attack he drew the bed-clothes over him with the left hand, talked freely and replied to questions, looked me straight in the face, and said, "I don't know you." Left side of face and ear hot and engorged from vasomotor paresis; soles of feet exquisitely tender; plantar reflex; and cremasteric both in very great excess. Later on through the day whenever he attempted to protrude the tongue left facial convulsions occurred.

I have recorded a similar case in which convulsions of the right side of the body with aphasia were twice induced by a strained mental effort.²⁷ This was also an instance of pro-

²⁷ *Textbook of Mental Diseases*, p. 256.