Deposits of iron in the liver and kidneys : with remarks on the demonstration of iron in tissues / Sheridan Delépine.

[*From the Proceedings of the Physiological Society*, May 10, 1891.]

SHERIDAN DELÉPINE:—1. *Deposits of Iron in the liver and kidneys, with remarks on the demonstration of Iron in tissues.*

The object of this communication is to attract attention,

1st. to the importance of using certain chemical reagents in definite proportions and in a definite sequence, for revealing Fe in the tissues;

2nd. to the fact that very slight changes of composition may obscure the reaction given by the iron compounds (so-called free iron) deposited in the liver and other organs under normal and abnormal circumstances.

I. I will limit my remarks to the Prussian Blue reaction, which is the most convenient though perhaps not the most universal in its application.

(1) Tizzoni[1] has recommended a fluid which is nothing else than a solution of Ferrocyanide of Potassium acidulated with Hydrochloric acid. This is a natural application of the well-known method of testing for Persalts of Iron.

(2) It appeared to me that the method although good in principle usually suffered from the mode of its application. If a large quantity of Ferrocyanide of Potassium be added to a small quantity of a Ferric salt in the presence of an insufficient quantity of Hydrochloric acid, no precipitate of Prussian Blue is produced, and if the disproportion be sufficiently marked a pale green instead of a deep blue colour is obtained. If, on the contrary, to a small quantity of a Ferric salt a small quantity of Ferrocyanide of Potassium be added, even a small proportion of Hydrochloric acid will cause an abundant precipitation of Prussian Blue.

The possibility of a variation in the amount of iron contained in the organs tested is not taken account of by Tizzoni, and the result is that with his fluid, tissues containing very notable quantities of iron very often give no reaction at all.

(3) To overcome these difficulties it was evident that one should in testing for iron

[1] Stirling, *Outlines of Practical Histology*, p. 243 (1890).

1st. avoid any excess of Ferrocyanide of Potassium;

2nd. use an excess of Hydrochloric acid (very dilute).

This object can be easily attained by placing the specimens tested

1st. in a solution of Ferrocyanide of Potassium (5 to 10 per cent.);

2nd. in water to remove the excess of Ferrocyanide of Potassium, (this of course is not necessary if the solution of Ferrocyanide is a weak one);

3rd. in a large quantity of a weak solution of Hydrochloric acid (0,5 to 1 per cent.), where it can be left from a few minutes to a few hours.

In specimens thus prepared the iron reaction is always clear, provided the iron be in certain states of combination.

II. (1) The iron may however be present and yet give a very poor reaction. This was first indicated to me by a case in which the kidneys contained a large amount of so-called free iron whilst there was apparently little or no trace of it in the liver.

(2) Every one knows that preparations kept in solution of chromic salts give the iron reaction very badly even when iron is pretty abundant in them, and that the same preparations kept in spirit on the contrary react very well. I thought that it might perhaps be possible to find some fluid which would free the iron still further from its organic connections. Weak Hydrochloric acid seems to act in this way. Artificial gastric juice acts more quickly still but unfortunately seems to remove the iron more rapidly than it does the protoplasmic elements of the cells. The fluid, however, which gave me the best results was a mixture of two parts of proof spirit with one part of glycerine. This fluid is also free from the objections that may on chemical grounds be offered against the use of Hydrochloric acid.

(3) When preparations containing iron are left in such a mixture from 12 to 24 hours and then treated as described above, the Prussian Blue reaction is generally rendered much more distinct than it is when the organs are fresh from the body—this improvement continues to increase for one or two months, but after this the reaction remains stationary or possibly begins to decrease slightly.

(4) Hæmoglobin and the pigments usually associated with or derived from it do not seem to be altered by this treatment, in fact the reaction is sometimes best obtained in parts (of the liver for instance) which seem to be hardly pigmented at all before the application and action of the reagents.

III. The series of specimens brought before the Society claims

interest, not only on account of its demonstrating the effects of the methods I have just described, but also because it shews that there may be very distinct differences in the state of combination of the so-called free iron found in organs in the case I have alluded to (a doubtful case of Pernicious Anæmia). I question whether this difference could have been proved by the use of ordinary chemical reagents, since the weakest among them would have certainly brought about alterations of composition proportionally so considerable as to obscure entirely such minor differences as must have existed in this case.

Immediately after removal of the organ from the body the kidney gave unmistakeable proof of its containing a large amount of so-called free iron. The liver gave no, or a very doubtful iron reaction. By leaving the organs for a few days in alcohol and glycerine, the iron reaction became a little brighter in colour in the parts of the kidney which were pigmented from the first but, what is more interesting, iron was revealed, and found to be abundant, in the portal zone of the liver which originally seemed to contain hardly any pigment, and gave practically no iron reaction[1].

As it is shewn by the behaviour of the various pigments of the body that the more intimately iron is bound up in organic combinations the more difficult it is to demonstrate the presence of that metal by processes which do not imply complete breaking up of these compounds, it seems only logical to assume that in this case the iron was a little freer in the kidney than in the liver. As the kidney did not seem to contain any marked amount of the pale or colourless iron compound which after a time was found to be present in the liver and vice versa, it seems evident that the iron contained in the liver was not in the same state as that which was being excreted from the body by the kidney. On the other hand the pale compound found in the liver and capable of yielding the iron reaction after the action of such a weak agent as a mixture of alcohol and glycerine was evidently an intermediate product between those colourless albuminates which do not yield the iron reaction before their organic connections have been artificially broken up, and those looser compounds which are found accumulated in the liver a certain number of hours after a meal or in certain diseases (such as pernicious anæmia).

[1] In the kidney the body containing iron was in the form of brownish yellow granules quite distinct even before the application of the Prussian Blue test.