THE REDUCTION OF FERRIC CHLORIDE BY SURVIVING ORGANS

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INTRODUCTION

In 1906 one of us showed¹ that on perfusing the surviving kidney with the gelatine and Prussian blue injection-mass, it was possible to obtain artificial urine in which the pigment was completely reduced to the leuco condition.

We were anxious to repeat this method with the kidney, and to employ it in the case of the liver in order to obtain, if possible, artificial bile, in which some reduced material might similarly be expected. In the first series of experiments we used the surviving liver of the cat just killed by bleeding; and we employed a solution of ferric chloride for two reasons:— (1) Its viscosity is so much less than the gelatine mixture; and (2) Because we had previously shown² that the reductase contained in the press-juice of liver reduced ferric chloride to the ferrous condition.

EXPERIMENTAL

(a) *Cat's Liver*

The technique of one experiment with the liver will suffice to show the method. A fine glass cannula filled with the solution of ferric chloride (0.25 per cent.) was inserted into the portal vein the instant the animal was dead. A similar cannula was tied into the gall-bladder, which contained a little viscous yellow bile. The opened abdomen was kept warm with pieces of cotton wool wrung out of hot water. From a burette, connected with the cannula by a rubber tube, a quantity of ferric chloride solution was allowed to run into the liver, which, in a few minutes, became fairly turgid. Discoloured blood in due time began to flow from the right external jugular vein, and was collected in a small glass flask. After the ferric chloride

1. Harris, D. Fraser, *Bio-Chemical Journal*, I, 355, 1906.

2. Harris, D. F., and Creighton, H. J. M., *Proc. Roy. Soc.*, 1912.

solution had displaced nearly all the blood from the liver, and had remained in contact with the liver tissue for twenty minutes, the liquid was squeezed out of the gall-bladder into the cannula which was removed from the organ, and the entire liver was rapidly excised while still warm. The liver was then cut to pieces in a glass funnel, through which a sanguineous, turbid fluid dripped. While the animal was being bled to death, some of its blood was collected and defibrinated, to be used in the control observations, as detailed below.

The following liquids were examined:—

- (1) Drippings from the cut liver (coloured red by blood), amounting, with washings, to 18 c.c.
- (2) 2 c.c. of bile from the gall-bladder.
- (3) 20 c.c. of blood (considerably diluted with iron solution) from the jugular vein.

Liver Drippings.—Before testing for iron, it was necessary to remove the haemoglobin from the solution, so as to get rid of the red colour. It was not practicable to precipitate the haemoglobin by heating, owing to the fact that, at the temperature of precipitation, ferric chloride is readily reduced by blood to the ferrous condition. In consequence of this, the haemoglobin was precipitated at room-temperature with dilute hydrochloric acid, which also served the purpose of breaking up any iron-protein complexes that might be present.¹ It was found when hydrochloric acid was slowly added to a solution containing blood, that, at first, the dark brown, soluble acid-haematin was formed, and that further addition of acid was necessary to precipitate the haemoglobin and blood-proteins.

To the 18 c.c. of liver drippings, 2 c.c. of dilute hydrochloric acid were added, and the precipitated haemoglobin and blood-proteins separated by filtration. The colourless filtrate was divided into several parts and tested for ferrous and ferric iron. *With potassium ferricyanide the colourless filtrate instantaneously turned to a deep green-blue colour and, after a few minutes, a blue precipitate was noticeable.* On the other hand, potassium ferrocyanide gave only a very faint blue coloration on being added to the filtrate.

As a control, the following experiment was carried out. To 10 c.c. of the defibrinated cat's blood, 1 c.c. of 0.25 per cent. ferric chloride solution was added. The mixture was then placed in a thermostat and kept at 38° for twenty minutes, after which the haemoglobin was precipitated with dilute hydrochloric acid, and removed by filtration. The colourless filtrate gave a deep blue

1. Creighton, H. J. M., *Trans. N. S. Inst. Sci.*, XIII, 61-75, 1912.

precipitate with potassium ferrocyanide, but with potassium ferricyanide the colour was yellow, *without the slightest trace of blue or green*. At the end of three-quarters of an hour the colour was still unchanged, but at the end of one and a half hours it had become slightly green, while after three hours the mixture was distinctly green and a slight precipitate had formed. This control experiment was repeated with the same results.

No ferrous iron was found to be present in the ferric chloride used in these experiments.

Bile.—The 2 c.c of bright yellow, viscous bile, to which a few drops of hydrochloric acid had been added, were diluted to 5 c.c with distilled water, and the solution divided into two parts. These were tested with potassium ferrocyanide and potassium ferricyanide; iron in both the ferric and the *ferrous* condition was found to be present.

Blood from Jugular Vein.—The haemoglobin was precipitated with dilute hydrochloric acid, and, after separation by filtration, potassium ferrocyanide and potassium ferricyanide were added to different portions of the clear filtrate. Both ferric and *ferrous* iron were found present.

(b) *Lamb's Kidney*

The technique for the kidney was briefly as follows:—The kidney was taken from a lamb just killed by bleeding was brought at once from the slaughter-house to the laboratory in the water-jacket of a calorimeter, which was filled with water at 42°. Within three-quarters of an hour of death, we fixed cannulae in the renal artery, the renal vein and the ureter. We perfused ferric chloride solution (0.25 per cent.) with a considerable head of pressure, and obtained a little sanguineous liquid from the renal vein and a large amount of a perfectly clear, pale yellow liquid from the ureter.

The following liquids were examined:—

(1) Blood from the Renal Vein.

(2) Artificial Urine from the Ureter.

Blood from Renal Vein.—The blood collected, which amounted to about 5-6 c.c., was treated with dilute hydrochloric acid, and the precipitated haemoglobin and blood-proteins filtered off. Portions of the clear filtrate were then tested with potassium ferrocyanide and potassium ferricyanide. The former reagent gave a deep blue precipitate, and the latter a pale green coloration, thus showing that some of the ferric chloride had undergone reduction.

Artificial Urine.—The artificial urine, which was somewhat lighter in colour than the ferric chloride solution used for perfusing the kidney, was acidified with hydrochloric acid and divided into two parts. On adding potassium ferrocyanide to one part, a deep blue coloration was obtained; while the other part gave, with potassium ferricyanide, a light green coloration, perceptibly deeper than in the case of the venous blood.

CONCLUSIONS

1. The foregoing experiments prove that ferric chloride is reduced to the ferrous condition, both by the surviving liver and the surviving kidney. In the light of our previous work¹ on the reductase of the press-juice of these organs, we believe that reductase is active in these intact surviving organs.

2. The fluid which runs through the blood-vessels of the organs is less reduced than that which is excreted into the gall-bladder and ureter, respectively. This is precisely what, *a priori*, we would expect, since the liquid flowing from the emergent vein has not come into contact with the living cells of the parenchyma, whereas the fluid found in the gall-bladder and the ureter has passed through the living cells of the hepatic tissue and renal tissue, respectively. This latter fluid has had greater opportunities of being brought within the sphere of action of the reductase.

3. The degree of reduction by the living cells is more perfect in the liver (of cat) than in the kidney (of lamb). This may be partly explained by an absolute difference in the intensities of the reduction in these two classes of organs; partly, possibly, by the fact that the liver was that of a carnivore, the kidney that of a herbivore; and partly by the fact, in the case of the artificial urine, that the perfusing liquid passed into the ureter very quickly, in consequence of which it was only for a very short time within the sphere of action of the reductase. From our previous work on the reductase of liver and kidney,¹ we are inclined to think that the supposition as regards the difference in reduction intensity is, in itself, correct.

4. The reductions studied are quite distinct from reductions in the presence of non-living organic matter.

1. *Loc. cit.*