THE INFLUENCE OF PROTOPLASMIC POISONS ON 'REDUCTASE'

By D. FRASER HARRIS, M.D., D.Sc., *Professor of Physiology and
Histology in the Dalhousie University, Halifax, N.S.*

(Received December 16th, 1911)

Continuing my investigations¹ into the reducing endo-enzyme, I have examined a number of 'protoplasmic poisons' to see whether they inhibited the activity of tissue reductase.

Waller² lately emphasised the necessity of distinguishing between the action of a poison upon protoplasm and its action on enzymes. He pointed to chloroform killing the laurel leaf but liberating instead of restraining an enzyme in the leaf which evolves hydrocyanic acid. The poison studied must not, of course, by itself bleach any of the pigments used in this research, viz., soluble Prussian blue, methylene blue and sodium indigo disulphonate. This condition eliminated such a poison as hydrocyanic acid, for instance, so that I need only record results with those as follow:—

Chloroform. Five drops of chloroform added to 5 c.c. of sheep's liver juice in presence of 5 c.c. of 0.06 per cent. of Prussian blue exerted only a very slight inhibitory action.

Chloroform has, however, the property of altering the physical state of the press-juice as to make it much less miscible with watery liquids so that it thus removes the pigment from the sphere of action of the reductase.

To vary the conditions, I perfused a sheep's kidney, previously washed till blood-free, with 1 per cent. sodium chloride having 10 per cent. chloroform in it. As soon as the vessels had been traversed by this mixture, the kidney became of a soft, 'dead white' appearance, and was also more friable.

1. This Journal, Vol. V. p. 143, 1910.
2. Proc. Phys. Soc., June 18th, 1910.

The glycerine extract of this chloroform-killed kidney reduced Prussian blue, but much more slowly than did the juice of a control of normal kidney.

The general conclusion come to was that chloroform did not inhibit the enzyme, but tended to coagulate the cell-proteins in which the enzyme was fixed and immobilised.

Sodium fluoride. A 0.5 per cent. solution was used. Employed against a control, this substance certainly did not seem to act as an inhibitor of the reducing enzyme. The solution was noticed to be distinctly alkaline, so that the bleaching might have been due to that condition.

Accordingly some sodium fluoride solution was made very slightly acid, and added to a mixture of glycerine extract of sheep's liver and Prussian blue. This mixture was well reduced, and the leuco compound readily restored by treatment with hydrogen peroxide. Sodium fluoride does not *per se* inhibit reductase.

Nitrobenzene. This is a powerful protoplasmic poison without reducing action on the three pigments. A glycerine extract of sheep liver juice was used with this poison.

After twelve hours in the water bath at 40° C. the contents of the tube with the poison and the control tube were both well reduced; the laboratory notebook has the entry: 'if anything, the tube with the nitrobenzene is the whiter.' This observation was repeated several times, so that I think we may infer that nitrobenzene is not a poison to 'reductase.'

Formalin (10 per cent. solution). This poison does not reduce Prussian blue although it precipitates it. Some kidney press-juice and Prussian blue were mixed with 5 c.c. of the formalin solution and, along with a control tube, were kept in the water bath at 40° C. overnight. Next morning the control tube was almost white, while the tube with the poison was still blue. Suspecting acidity, I found that this formalin was acid both to litmus and to phenolphthalein. When a little formalin was the very slightest over-neutralised by some N/10 sodium hydrate, and added to kidney juice in presence of Prussian blue, the pigment was bleached. It would appear that the reductase was previously inhibited by the acidity of the formalin and not by its poisonous property, whatever that may be.

From these experiments the general impression seems to be that acidity is the inhibitant factor rather than the toxicity. Hydroxylamine sulphate, for instance, a powerful protoplasmic poison, is so acid in reaction that the fact that it prevents any fading of Prussian blue in presence of tissue-juice is not to be taken as a proof that hydroxylamine sulphate inhibits the reducing endo-enzyme.

The expenses involved in this research were met by a grant from the Government Grant Committee of the Royal Society.