CX. A NOTE ON THE PRESENCE OF GLUTATHIONE IN THE CORPUSCLES OF MAMMALIAN BLOOD.

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The work to be described was undertaken with the intention of investigating the optically active substances of blood other than glucose.

It was stated by Winter and Smith [1922] that the optical rotation of a concentrated protein-free filtrate of mammalian blood was initially less than that calculated from the amount of glucose estimated to be present. This difference they attributed to the presence of "an unstable modification of glucose."

The writer observed that protein-free filtrates of the corpuscles of sheep and ox blood were free from glucose but contained a laevo-rotatory substance in significant amounts and that its rotation decreased with increase in the hydrogen ion concentration of the solution, thus affording a possible explanation of the phenomena recorded by Winter and Smith.

It was decided to attempt to isolate and identify this substance. The process was necessarily tedious since the optical properties threw little light on its chemical nature.

After numerous attempts with a variety of precipitating agents the following method was employed.

Four litres of sheep's blood were freed from protein with tungstic acid [Folin and Wu, 1919], and the precipitate well washed with cold water. To the united filtrate and washings were added 35 cc. of 10 % mercuric sulphate in 5 % sulphuric acid for each litre of fluid. The precipitate was filtered off and well washed with cold water. It was decomposed with hydrogen sulphide and the sulphuric acid exactly removed with baryta. The filtrate was concentrated under diminished pressure to 50 cc. and enough sulphuric acid added to make the solution semi-normal. Phosphotungstic acid dissolved in semi-normal sulphuric acid was added till the maximum precipitate was obtained. The precipitate was filtered off, well washed, and decomposed with baryta: the barium tungstate was removed by filtration and the excess baryta removed with sulphuric acid. The solution was concentrated to a syrup and on addition of alcohol a white powder was precipitated.

The specimen thus obtained proved to contain both "neutral" sulphur and amino nitrogen. After reduction it yielded the nitroprusside reaction. It had the same melting point as glutathione from other sources and when mixed with a specimen of pure glutathione from yeast the melting point was unchanged. After acid hydrolysis characteristic crystals of glutaminic acid hydrochloride were obtained.

No trace could be found of it in sheep's plasma, which is in accordance with Arnold's [1911] and Hopkins' [1921] earlier experiments using the nitroprusside reaction. Hopkins found that phosphotungstic acid precipitates gluthathione if the latter is in a moderately concentrated solution. He accordingly worked at a low concentration, whilst in the present case the solution was previously concentrated in order to assist the precipitation.

Subsequent experiments indicate that it is present in the corpuscles of sheep, goats, rabbits and rats in the reduced form. Its presence was apparently overlooked by Tunnicliffe [1925].

The yield of 0·05 g. per litre obtained is probably considerably below the actual concentration, since the methods of precipitation involve a considerable loss.

It may be of interest to note that both cysteine and reduced glutathione have the power to convert methaemoglobin into oxyhaemoglobin and to reduce the latter to haemoglobin *in vitro*. This may afford an explanation of the rapid conversion of methaemoglobin into oxyhaemoglobin in the animal body.

Summary.

Glutathione has been identified as a constituent of the red corpuscles of sheep's blood.

It apparently exists in the corpuscles of many animals in the reduced form.

It is apparently the chief optically-active constituent of de-proteinised blood other than glucose and as such is responsible for the effects recorded by Winter and Smith and others and quoted as evidence in favour of the presence of an unstable modification of glucose.

It also probably accounts for much of the "neutral" sulphur of blood.

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