XCIX. A NOTE ON THE ISOLATION OF ERGOTHIONEINE FROM BLOOD.

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THE methods employed by Hunter and Eagles [1925], by Benedict, Newton and Behre [1926], and by Benedict and Newton [1929] for the isolation of ergothioneine from blood are somewhat involved and expensive to apply. The authors have devised a technique (based on Hopkins's [1929] method for glutathione) which yields about 85 % of the ergothioneine present. The method involves three stages.

(1) The extraction of the blood with dilute acid, and the subsequent coagulation and removal of the proteins.

(2) The concentration of the solution *in vacuo* and aeration of the concentrate.

(3) The precipitation and washing of the cuprous compound of ergothioneine, and its decomposition with H_2S , followed by concentration of the aqueous solution.

The whole process can conveniently be carried out in three days, and, if it be desired, glutathione can be recovered from the liquid filtered from the cuprous compound of ergothioneine.

EXPERIMENTAL.

5 litres of pig-blood were poured into an equal volume of dilute sulphuric acid (0.2 % by volume) and the whole warmed to 80°. The protein was filtered from the hot mixture on large Büchner funnels, the precipitate squeezed in a large filter press, and the expressed liquid added to the main volume of the solution. The volume of the total filtrate was about 8 litres. 1 litre of "dialysed iron" (B.D.H.) was added to the solution. The "dialysed iron" removes traces of protein and of haematin and appears to remove a so far unidentified substance, present in minute traces in pig-blood, which forms an insoluble compound with cuprous oxide. The mixture was filtered through pleated filter-papers and the filtrate evaporated *in vacuo* to a volume of about 1 litre and again filtered, to remove traces of uric acid.

The resulting solution contains reduced glutathione as well as ergothioneine. Reduced glutathione reacts with cuprous oxide, but oxidised glutathione does

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not [Hopkins, 1929]. The solution is brought to p_H 7·4–7·6 with alkali and vigorously aerated till the nitroprusside reaction for the sulphhydryl group of glutathione disappears. (Ergothioneine does not give a nitroprusside reaction.) Sulphuric acid is added to 0·5 *N* concentration and the solution filtered, and warmed to 60°. A dilute aqueous suspension of cuprous oxide [Hopkins, 1929] is added in small amounts at a time, the liquid being vigorously stirred. The cuprous compound of ergothioneine falls out as a ropy precipitate which can be collected on a glass rod. Cuprous oxide is added till the precipitate acquires a faint purplish tint, but there is no danger in adding a slight excess of copper oxide. The cuprous compound of ergothioneine, unlike that of glutathione [Hopkins, 1929; Pirie, 1930], does not dissolve in presence of slight excess of cuprous oxide. The precipitate is allowed to settle and the supernatant liquid decanted off through a filter. The solution contains oxidised glutathione, which can be isolated by the usual methods. The precipitate of cuprous ergothioneine is then washed, best on the centrifuge with 60 % alcohol. About 10 washings are normally sufficient to remove sulphate. The copper derivative is then suspended in water and decomposed with H_2S . The copper sulphide must be well washed with hot water, and the combined filtrates are then evaporated *in vacuo* to about 10 cc. A few drops of concentrated hydrochloric acid are added, and the solution is further concentrated in a desiccator to 1 or 2 cc., when ergothioneine hydrochloride crystallises out, m.p. 208°. It may be converted to the free base (m.p. 262°) by the method of Benedict, Newton and Behre [1926]. (Found: N, 17·98; S, 13·91 %. $C_9H_{15}O_2N_3S$ requires: N, 18·34; S, 13·97 %.)

Hunter [1928] devised a modification of the Pauly diazo-test for histidine which, he claims, is specific for ergothioneine, and which permits of its quantitative estimation in blood. The total amount of ergothioneine was determined by Hunter's method. In general the yield of ergothioneine obtained by the above-described method was about 85 % of the total present.

SUMMARY.

A method is described of isolating ergothioneine from pig-blood. The yield is about 85 %, and the process is successful when the initial concentration of ergothioneine is as low as 20 mg. per litre and the earlier methods cannot be applied.

We have great pleasure in thanking Sir F. G. Hopkins for his interest in this work.

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