LV. A NOTE ON THE DETERMINATION OF ERGOTHIONEINE IN BLOOD FILTRATES.

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SOME points in connection with an article which has recently appeared by Salt [1931] on the ergothioneine content of human blood seem to call for comment. Methods for the determination of ergothioneine are still in the experimental stage and, until a method is available which has been proved to be really reliable, figures for the normal range of this compound in blood, and for its variation in pathological conditions, cannot be established. Under these circumstances any comments which may help to clarify the subject seem advisable at the present time.

A method for the determination of ergothioneine (then called thiasine) was described by Benedict, Newton and Behre [1926] which depended upon the amount of colour formed when sodium cyanide and the regular uric acid reagent [Benedict, 1922] were heated with the silver residue remaining after the indirect removal of uric acid by means of the silver lactate-acid sodium chloride treatment of whole blood-filtrates.

Subsequently a second method was described by Behre and Benedict [1929] which involved the determination of ergothioneine in the same silver residue but which utilised sodium hydroxide as alkali for the development of colour with the uric acid reagent, the amount of cyanide used being reduced to the minimum necessary for the solution of the silver residue. The colour reaction in this case takes place at room temperature. This method was believed to be more accurate than the earlier one because (1) the use of hydroxide (as alkali) instead of cyanide increases the specificity of the reaction for ergothioneine as compared with uric acid, and (2) lower ergothioneine figures were obtained from whole blood filtrates by the second method, while at the same time added ergothioneine was completely recovered. The fact that lower blood figures were found by the second method led us to assume that the silver residue contained some substance other than uric acid (and almost certainly not glutathione) which was capable of reacting in the presence of cyanide but not where sodium hydroxide was employed as the alkali. Although we still feel that the method just referred to is preferable to any of the others which we have tried, we are well aware that in its present form it has some disadvantages. The presence of even the small amount of cyanide used for the solution of the silver residue

reduces the depth of colour given by ergothioneine with sodium hydroxide, so that many of the blood filtrates give a final colour which is so light that the colorimetric reading must be made with the greatest care. It is also necessary to add an amount of cyanide to the ergothioneine standard exactly equal to the amount added to the silver residue¹. No satisfactory standard other than a solution of pure ergothioneine has been found for use in this method. We are hoping shortly to be able to overcome these difficulties.

The procedure for the ergothioneine determination which has recently been described by Salt [1931] is essentially identical with our first method [Benedict, Newton and Behre, 1926] except that the determination is carried out on filtrates from the corpuscular residues obtained by the preparation of unlaked extracts according to Folin's directions [1930]. Salt [1931] does not specify his reason for using such filtrates (preparation of which involves two precipitations and a re-solution of one of these precipitates) in preference to using the filtrate from whole blood. Indeed, he presents figures which show that practically all the chromogenic substance is present in the corpuscles, so that double precipitation of the blood seems to have no special value. According to Salt's table, the amount of the chromogen present in the plasma fraction (the "unlaked extract") usually approaches zero and is never equivalent to more than 0.2 mg. of uric acid (per 100 cc.). This amount seems hardly great enough to justify the cumbersome procedure involved in the use of the corpuscular centrifugate. Nor is the amount of colour given by the unlaked plasma fraction great enough to warrant the assumption that it represents the unknown substance present in the silver residue from whole blood which, as we have stated, reacts in presence of cyanide and not with hydroxide. Taking this into consideration one is led to question whether the ergothioneine figures reported by Salt represent only ergothioneine or whether they may not also include a part of this unidentified, cyanide-reacting substance. The same doubt is cast upon other ergothioneine figures which have been based upon methods where cyanide is used [Rockwood, Turner and Pfiffner, 1929].

Salt's paper also includes a comparison of the blood-ergothioneine obtained after the use of different protein precipitants, taking the "direct" uric acid determination as an indication of the amount of ergothioneine present. According to his figures much less blood-ergothioneine was found in filtrates after precipitation with molybdic acid than after precipitation with either tungstic or tungstomolybdic acid. Figures for the recovery of ergothioneine added to blood are not given, but it is stated that added ergothioneine was quantitatively recovered from tungstomolybdic acid filtrates. The results with regard to molybdic acid are at variance with figures reported by Behre and Benedict [1929], and Benedict and Newton [1929], which showed that in the 15 bloods

¹ In the original directions for this method [Behre and Benedict, 1929], it was stated that 6 drops of the cyanide were to be added to the standard. Since more than 6 drops are sometimes necessary for the solution of the silver residue, this statement should be corrected to the effect that the same amount of cyanide is added to the standard as was added to the unknown.

examined ergothioneine added to blood was completely recovered after precipitation with molybdic acid.

Salt does, however, agree with our finding that tungstomolybdic acid causes no loss of ergothioneine. This protein precipitant is the one now employed in this laboratory preliminary to determination of any or all of the non-protein constituents of blood [Benedict, 1931].

With such uncertainty as to the methods at present available for the determination of ergothioneine in blood, it is obvious that no great reliance can be placed on the figures so far obtained. It is, however, interesting to find that Salt's figures corroborate our earlier findings [Benedict, Newton and Behre, 1926] to the effect that the ergothioneine content of diabetic blood tends to be higher than the normal. The average of Salt's figures for diabetic blood is nearly 3 mg. (38 %) above his average for normal blood. A quite similar relationship holds between Salt's average figure for ergothioneine in blood from diabetics as compared with blood from nephritics. It may also be noted that Salt's minimal and maximal figures for ergothioneine are definitely higher in diabetic than in normal blood.

A single determination on diabetic blood reported by Rockwood, Turner and Piffner [1929] also gave a slightly higher figure for ergothioneine than the normal figures reported by these workers. As Salt has pointed out, the range of values for ergothioneine in both normal and pathological blood appears to be so great that slight differences should not be too heavily stressed. That all the results on diabetic blood so far reported have tended in the same direction appears however to be of some significance. Apparently Salt was not familiar with the fact that we had already [Benedict, Newton and Behre, 1926] pointed out a definite tendency to higher ergothioneine figures for diabetic bloods. Had he known of our findings in this connection, he might have been less cautious in concluding with us that in diabetes there is tendency to an increased ergothioneine content of the blood.

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