CCXVI. SOME LIMITATIONS OF THE CARIUS DIGESTION FOR THE MEASUREMENT OF CHLORIDE IN BIOLOGICAL MATERIAL.

BY FREDERICK WILLIAM SUNDERMAN
AND PRISCILLA WILLIAMS.

From the John Herr Musser Department of Research Medicine, University of Pennsylvania and the Pennsylvania Hospital, Philadelphia.

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IN the quantitative determination of chloride of desiccated biological tissues, particularly those containing fats, it has been amply demonstrated that complete recovery is not obtainable by the method of open Carius digestion with acid. On the other hand the use of preliminary alkaline digestion followed by the open Carius method has been shown by one of us [1933] to give quantitative recovery.

The failure to recover the chloride completely by the Carius method has been interpreted by some, particularly by Morris and Morris [1930; 1932] as due to an actual loss of chloride in some volatile form during the drying process. We have shown that this interpretation is incorrect since we have reported [1931] that if desiccated blood be allowed to stand in contact with water before the Carius digestion, almost complete recovery of chloride results; and more recently one of us has shown [1933] that subjecting dried tissues to preliminary alkaline digestion yields complete recovery as compared with established methods of analysis. The confusion between "diminution in chloride measurement" and an actual loss of chloride persists in the recent article by Norris and Ampt [1933] who have failed to recognise that our experiments indicated not a loss of chloride but rather a failure to measure it.

It was suggested by us that traces of halide may distil off during the digestion of blood and tissues with AgNO_3 and HNO_3 , though in quantities insufficient to account for the diminution in chloride measurement which we reported in the analysis of desiccated biological materials. The following experiments were devised to measure the amount of halide lost during the Carius digestion, using blood as a test material.

Samples of wet and dried blood were digested with AgNO_3 and HNO_3 in a pyrex flask connected by ground glass joints to the digestion flask of a Thompson-Oakdale apparatus¹. The fumes given off from the digestion mixture were passed through hot concentrated H_2SO_4 in the digestion flask of the Thompson-Oakdale apparatus, and after having passed through quartz tubing maintained electrically at cherry-red heat they were collected in a solution of Na_2HAsO_3 . The residual fumes following the Carius digestion were drawn through the Thompson-Oakdale

¹ In some of the experiments a similarly modified Willard-Thompson apparatus was employed.

apparatus in a stream of chloride-free air. The Na_2HAsO_3 solution was neutralised with HNO_3 , and the halide precipitated with AgNO_3 . The results of the analyses are given in Table I. The estimations of the halide recovered from the fumes were made by comparing the turbidity of the unknown with that of freshly prepared suspensions of AgCl . By comparison of turbidity we were able to estimate any chloride that might be lost up to 3 *mM* per litre of blood within $\pm 0.1 \text{ mM}$.

Table I.

Material	Remarks	Maximum halide re- covered from fumes from Carius digestion. Milli-equiv./l. wet sample	Chloride analyses. Milli-equiv./l. wet sample
Ox blood 16	Wet samples dried in flasks at 110° for 24 hours	0.2 0.5	80.1 66.8
Ox blood 18	Wet samples dried and subjected to preliminary alkaline digestion	0.3	—
Human blood	(a) Wet samples	0.5	—
	(b) Dried and finely pulverised samples	1.0	—
	(c) Samples dried in flasks	1.8	—
NaCl sol. 100 milli-equiv./l.	—	0.1 (same as reagent blank)	—
Human blood	(a) Wet samples	—	83.2
	(b) Dried samples subjected to preliminary alkaline digestion	—	83.8
	(c) Wet samples digested in flasks connected with stoppered re- flux condensers	—	83.0
	(d) Dried samples digested in flasks connected with stop- pered reflux condensers	—	68.8

It will be seen from Table I that the minute amount of halide which we were able to recover from the fumes during the Carius digestion of blood accounts only to a very small extent for the failure to measure chloride completely by the direct Carius method with dried blood. Moreover, when dried blood was subjected to direct Carius digestion in Erlenmeyer flasks connected by ground glass joints to stoppered reflux condensers, failure to measure chloride completely was still experienced. These studies suggest that failure to measure the chloride completely in desiccated biological tissues must be attributed to other factors rather than to a loss of chloride.

We are in agreement with Norris and Ampt as to the desirability of alkaline digestion of certain biological materials before proceeding with the measurement of chloride. In the case of wet blood or serum, however, preliminary alkaline digestion was found unnecessary.

Our studies demonstrate that in some biological materials chloride is not completely measured by the direct open Carius method. Preliminary leaching with water in some instances provides almost complete recovery of the chloride; preliminary digestion with alkali is completely effective and is, therefore, the

procedure of choice in dealing with such materials. We have been unable to demonstrate that this failure to measure chloride by the direct open Carius method is due to a loss of chloride in amounts greater than 1.8 *mM* per litre of blood.

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