XXXVI. A NOTE ON THE ESTIMATION OF CHLORINE IN MILK.

BY ALFRED DUDLEY HUSBAND AND WILLIAM GODDEN.

From the Rowett Research Institute, Aberdeen.

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AUSTIN AND VAN SLYKE [1920] have shown that the Van Slyke-Donleavy [1919] method for the determination of plasma chlorides, when applied to whole blood, gives results too high by 30 to 40 %. If, however, the protein is precipitated by picric and nitric acids, and the protein-free filtrate is treated with silver nitrate, a quantitative precipitation of the total chloride of the whole blood is obtained and the final titration may be carried out as in the Van Slyke-Donleavy method.

Sisson and Dennis [1921], employing the Van Slyke-Donleavy method, with slight modifications, for the determination of chlorine in human milk, claim to have obtained results essentially similar to those obtained by fusion of the milk with sodium carbonate, to remove the organic material, and subsequent titration by the Volhard method. We have investigated the suitability of these two methods for the determination of chlorine in milk and have checked our results by means of Volhard titration after ashing the milk with sodium carbonate or with lime.

It was found, in testing these methods, that it was advisable to use the modified picric acid solution suggested by Sisson-Dennis to obtain complete precipitation of the milk proteins. The technique as used by us was as follows.

(a) *Method of Sisson-Dennis.* To 10 cc. of milk measured into a small flask were added 20 cc. of 1.2 % picric acid solution containing 2 cc. of glacial acetic acid per litre. The mixture was well shaken and allowed to stand for 5–10 minutes. 10 cc. of *N*/10 silver nitrate were then added, the mixture was again shaken and filtered on a chlorine-free filter paper. 20 cc. of clear filtrate were measured off and titrated against standard ammonium thiocyanate solution, as in the Volhard method.

(b) *Method of Austin and Van Slyke.* To 20 cc. of milk measured into a small flask were added 40 cc. of the picric acid solution, and after 10 minutes the curd was filtered off on a chlorine-free filter paper. 30 cc. of clear filtrate were then treated with 10 cc. of *N*/10 silver nitrate, the mixture was shaken, again filtered and 20 cc. of the clear filtrate were titrated against the standard thiocyanate.

The Volhard method for making the final titration was adopted for the sake of convenience.

The results obtained by us do not agree with the findings of Sisson-Dennis, but are in entire agreement with the results obtained by Austin and Van Slyke on whole blood.

In all the milks we tested the figures obtained were too high unless the protein was first removed before adding the silver nitrate solution. A comparison of the percentage of chlorine found by the different methods is shown in Table I.

Table I.

No. of sample	Animal	Method of Sisson-Dennis Cl %	Method of Austin-Van Slyke Cl %	Method of ashing Cl %
1	Cow	0.1276	0.1028	0.1028
2	"	0.1276	0.1064	—
3	"	0.1347	0.1064	—
4	Goat	0.1808	0.1560	—
5	"	0.1454	0.1135	0.1135

The figures given in Table II indicate that when using the method of Sisson-Dennis the results are liable to vary if changes are made in the ratio of milk to silver nitrate used in the determination, whereas, by the method of Austin-Van Slyke, the percentage of chlorine found is independent of these changes provided that an excess of silver nitrate is present.

Table II. *Effect of varying the quantities of milk and silver nitrate.*

No. of sample	Amount of milk cc.	Amount of AgNO ₃ cc. N/10	Method of Sisson-Dennis Cl %	Method of Austin-Van Slyke Cl %
1	10	10	0.1276	0.1064
1	20	10	0.1383	—
1	10	20	0.1312	—
2	10	10	0.1347	0.1064
2	5	10	—	0.1090
2	20	10	—	0.1064

The only effect of varying the amount of picric acid is to change the rate of filtration. In view of the fact that the presence of the protein of milk gives values too high when determining the chlorine content of milk a few tests were carried out on a chlorine-free solution of caseinogen using the methods described above.

Table III shows that only by the method of Sisson-Dennis was any apparent chlorine content found. This would appear to be due to either adsorption or combination of some of the silver by the precipitated protein.

Table III. *Test of methods on chlorine-free solution of caseinogen.*

Amount of caseinogen in solution g.	Apparent Cl-content found by method of Sisson-Dennis g.	Cl found by method of Van Slyke	Cl found by ashing with lime
0.3	0.00354	Nil	Nil
0.3	0.00213	"	"

CONCLUSION.

To determine accurately the chlorine content of milk volumetrically by precipitation of the chlorine as silver chloride, it is essential that the protein be removed before the addition of the silver nitrate.

REFERENCES.

- Austin and Van Slyke (1920). *J. Biol. Chem.* **41**, 345.
Sisson and Dennis (1921). *Amer. J. Dis. Child.* **21**, 389.
Van Slyke and Donleavy (1919). *J. Biol. Chem.* **37**, 551.