Table 1. *Protein equivalents of a set of permanent turbidimetric standards*

(3 ml. of 3% sulphosalicylic acid plus 1 ml. protein solution.)

Polymer in standard (g.)	Protein equivalent of standard (mg./100 ml.)
0·150	6
0·225	10
0·235	15
0·275	20
0·300	27
0·310	35
0·325	42
0·375	50
0·400	60
0·42	75
0·45	85
0·48	100

Note. Each standard must be calibrated in terms of the sulphosalicylic acid-protein procedure, irrespective of the amount of polymer used. With the amount of polymer used here the turbidities are not in strict arithmetical ratio; but they fall approximately on a smooth curve.

By preparing a large number of standards it is possible to select those which show turbidities corresponding to protein concentrations of convenient selected amounts, e.g. 10, 20, ..., 90, 100 mg./100 ml.

standing with his back to a window and holding the rack of standards at eye level to be illuminated by daylight.

Calibration of the standards. This has been accomplished by preparing several dilutions of blood plasma whose protein content was accurately established by Kjeldahl determinations and treating them by the above procedure. Eight samples of normal blood plasma were used, and the average results for the Haslam & Squirrel standards are given in Table 1. The accuracy of the matched comparisons as judged by the Kjeldahl figures is about ±10%, i.e. the average observer would assign a value to a 50 mg./100 ml. protein solution of anything between 45 and 55 mg./100 ml.

SUMMARY

The sulphosalicylic acid procedure for the turbidimetric estimation of protein has been successfully used with the permanent Perspex standards of Haslam & Squirrell (1950). The method yields only approximate results (about ±10%); but is very useful for such purposes as the estimation of urine and C.S.F. protein in hospital laboratories. The standards are convenient to use, and appear to be permanent.

I am indebted to Mr J. Haslam for his ready co-operation and help in preparing the standards. My thanks are due to Miss V. Pash and Mr T. A. Webster for their valuable assistance in the calibration.

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A Simplified Silver Iodate Method for the Determination of Chloride

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A method for the determination of chlorides in the blood and other biological fluids has been described by Haslewood & King (1936) and by Sendroy (1937; preliminary communication 1935). These methods are based on the reaction

$$NaCl + AgIO_3 \rightarrow AgCl + NaIO_3.$$

The sodium iodate formed is made to react with potassium iodide, added subsequently, from which three molecules of titratable iodine are liberated for each atom of chloride originally present; the final titration is with thiosulphate with starch as indicator.

The Haslewood & King procedure involved the preparation of a protein-free filtrate by Somogyi's (1930) zinc hydroxide method, the addition of an ammoniacal solution of silver iodate to this filtrate, subsequent removal of silver chloride and excess silver iodate by filtration of the solution after acidification with sulphuric acid, and titration of a sample of this filtrate with thiosulphate solution after the addition of potassium iodide.

Sendroy's procedure involved the precipitation of the plasma proteins with a special tungstic acid solution, filtration, and the shaking of the filtrate with a specially prepared purified solid silver iodate. The reaction between the dissolved sodium chloride and the silver iodate is complete in a very few minutes, and the silver chloride and excess silver

iodate are then filtered off. A sample of the filtrate is treated with potassium iodide and titrated with thiosulphate.

Van Slyke & Hiller (1947) simplified the Sendroy method by adding the solid silver iodate to the mixture of diluted plasma with sodium tungstate and phosphoric acid before removing the proteins, and then, after shaking, filtered off the proteins and the silver chloride and extra silver iodate in one operation. This method is very convenient and accurate, but it requires the preparation of the special silver iodate reagent of Sendroy (1939).

A modification of the Haslewood & King procedure has been developed with a view to reducing to a minimum the number of technical operations involved, and to using ordinary commercial silver iodate, which is used as before in ammoniacal solution, instead of the specially prepared solid silver iodate reagent of Sendroy. The proteins, silver chloride and excess silver iodate are precipitated together and removed by a single filtration, as in the Van Slyke & Hiller procedure. The method is quick and accurate; and the results are in good agreement with those obtained in parallel estimations carried out by the Van Slyke & Hiller method and by the mercuric nitrate method of Schales & Schales (1941).

EXPERIMENTAL

Solutions

Ammoniacal silver iodate solution. The stock solution consists of 1·8% (w/v) silver iodate ($AgIO_3$) dissolved in N-NH_3 solution. The working solution is prepared fresh before use by adding an equal volume of N-H_2SO_4 to, for example, 5 ml. of the stock in a centrifuge tube, and mixing well, to precipitate the $AgIO_3$. After centrifuging and pouring away the supernatant, the precipitate is dissolved in 0·3 N-NH_3 equal in volume to that of the stock $AgIO_3$ solution taken. This precipitation and resolution ensure that the $AgIO_3$ used is free of soluble iodate.

N-*Ammonia solution.* 57 ml. conc. NH_3 (sp.gr. 0·882) to 1 l. with water.

0·3 N-*Ammonia solution.* 17 ml. conc. NH_3 (sp.gr. 0·882) to 1 l. with water.

Tungstate-phosphoric acid reagent. 4·2 g. sodium tungstate ($Na_2WO_4.2H_2O$) are dissolved in 1 l. of 0·15 M-H_3PO_4. The latter is prepared by making 10 ml. of conc. H_3PO_4 (sp.gr. 1·72) to 1 l. with distilled water.

Potassium iodide 2% (*w/v*).

0·005 N-*Sodium thiosulphate.*

Method

To 0·2 ml. of plasma is added 0·5 ml. of ammoniacal $AgIO_3$ solution, followed by 3·3 ml. of tungstate-phosphoric acid reagent. The mixture is shaken well and filtered. (It is convenient to use a 7 cm. Whatman no. 42 filter paper.) The filtrate (1·0 ml.) is treated with 1·0 ml. of 2% KI solution and then titrated with 0·005 N-$Na_2S_2O_3$ solution, using starch as indicator.

For determining the chloride in simple aqueous standard solutions the above procedure was found to be unsatisfactory, because of the presence of tungstate. For such solutions the method was modified by using 0·15 M-H_3PO_4 instead of the tungstate-phosphoric acid reagent, thus: to 0·2 ml. of standard solution, 0·5 ml. of ammoniacal $AgIO_3$ solution was added and then 3·3 ml. of 0·15 M-H_3PO_4, etc.

For whole blood the amount of sodium tungstate in the tungstate-phosphoric acid reagent should be doubled.

Table 1. *Comparison of chloride methods with standard solutions of sodium chloride*

Chloride present (mg. NaCl/100 ml.)	Chloride determined (mg. NaCl/100 ml.)		
	Present iodate method	Van Slyke & Hiller method	Schales & Schales method
305	305	305	301
	304	306	306
	305	307	306
450	448	454	449
	449	454	454
	450	455	454
607	607	603	—
	607	607	607
	—	607	—
750	746	745	744
	747	748	749
	748	748	755

Table 2. *Comparison of chloride methods with specimens of blood plasma*

Chloride determined (mg. NaCl/100 ml. blood)		
Present iodate method	Van Slyke & Hiller method	Schales & Schales method
618	617	618
618	610	618
618	616	612
595	593	585
598	594	591
600	595	591
602	601	596
605	600	607
605	604	612
607	604	612
608	607	—
626	625	629
628	626	633
628	627	633
631	628	—
371	367	369
372	368	379
373	368	379
377	371	389

RESULTS

The results obtained by this procedure for chloride analysis were compared with those obtained by two other methods of chloride determination, namely the iodometric method of Van Slyke & Hiller (1947) and

the mercurimetric method of Schales & Schales (1941). Tests were performed on both standard solutions and specimens of plasma. The results obtained are set out in Tables 1 and 2.

Results in the Schales & Schales method were calculated by comparison with a standard solution, the chloride content of which was 607 mg. sodium chloride/100 ml.

SUMMARY

A rapid and accurate method of estimating plasma chloride has been developed, using as a basis the silver iodate procedure of Haslewood & King (1936). The results obtained from this and the methods of Van Slyke & Hiller (1947) and Schales & Schales (1941) were in good agreement.

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Studies in the Biochemistry of Micro-organisms

82. USTIC ACID, A METABOLIC PRODUCT OF *ASPERGILLUS USTUS* (BAINIER) THOM & CHURCH

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The *Aspergillus ustus* group of Aspergilli, as defined by Thom & Raper (1945) in their *Manual*, includes only two species, *A. ustus* (Bainier) Thom & Church and *A. granulosus* Raper & Thom. *A. ustus* is regarded by Thom & Raper as a single species aggregate including a whole series of interrelated individual strains differing markedly in their general habit, colony coloration and other characteristics. Representatives of the *A. ustus* group are perhaps the most abundant of all Aspergilli in the soil.

In spite of the ubiquity of *A. ustus* little was known until recently of its biochemical activities. Kurung (1945) reported that culture filtrates from a strain of *A. ustus* grown on a modified Czapek-Dox medium containing potassium chloride (0·5 g./l.) had marked antibacterial activity. Hogeboom & Craig (1946), in a continuation of this work, isolated two crystalline metabolic products containing chlorine, $C_{21}H_{17}O_6Cl_3$ (m.p. 185–187°) and $C_{21}H_{18}O_6Cl_2$ (m.p. 214–216°). Doering, Dubos, Noyce & Dreyfus (1946), in a parallel investigation, described other chlorine-containing metabolites of the same strain of *A. ustus* and gave the name ustin to the predominant component. Ustin was shown to be identical with Hogeboom & Craig's substance melting at 185–187°, but was assigned the formula $C_{19}H_{15}O_5Cl_3$.

We have found that four different strains of *A. ustus*, when grown on Raulin-Thom medium, which does not contain any chlorides, yield culture filtrates which give very intense colours with ferric chloride varying in shade from almost pure purple to purple-brown. In contrast, no comparable colours are given by culture filtrates of three of the same four strains of *A. ustus* when grown on Czapek-Dox medium. The metabolic product responsible for the purple colour has been isolated in a pure state and we propose the name *ustic acid* for it, since it has not been described previously. Pure ustic acid was isolated in yields of about 0·5 g./l. of culture filtrate from the best strain, grown on Raulin-Thom medium: the other three strains gave smaller yields. Ustic acid, $C_{11}H_{12}O_7$, forms colourless, rhombic prisms, m.p. 169–170° (decomp.). It has no optical activity. It contains one methoxyl group and is a strong acid giving a colourless crystalline potassium salt, $C_{11}H_{11}O_7K$. An aqueous solution of ustic acid gives an intense, stable, purple colour with aqueous ferric chloride; immediately decolorizes bromine water without the formation of a precipitate; does not give a precipitate with Brady's reagent (2:4-dinitrophenylhydrazine in 2N-hydrochloric acid) except after standing for some hours; and, at room temperature, rapidly reduces Fehling's solution, copper acetate solution and neutral copper sulphate solution with the formation of cuprous oxide in each case. On boiling with 2N-sulphuric acid, ustic acid is not decarboxylated, but is converted, with the loss of one molecule of water, into a colourless crystalline com-