virus may reach rather high concentrations before the usual extensive neuronal damage appears and frank paralysis sets in. The highest concentration seems to be reached at the time of paralysis, which is presumably immediately after the bulk of the anterior horn cells have suddenly crumbled under the action of a certain rather high critical concentration of virus.

Superficially at least, the relationships seem to be analogous to those observed in bacteriophagy in that fairly large amounts of virus may be formed in the absence of apparent nerve cell damage and in that actual cell destruction may be a function of the concentration reached by the virus, as well as of the general level of susceptibility of the cells exposed to it. In other words, virus production and nerve cell damage do not altogether parallel each other. It may be that the amount of damage sustained by the nerve cells in any given region of the nervous system depends rather more on the concentration which a given virus is capable of building up than upon its invasiveness as such.

In the case of bacteriophages it is known that individual bacteriophages on serial passage eventually reach a certain concentration endpoint, either low or high, beyond which they cannot be increased. To us it seems possible that like some strains of bacteriophage, which despite serial passage remain of low titer and low lytic power, there are constantly low titer strains of poliomyelitis virus, which although not lacking in invasiveness may be unable to build up a concentration high enough to damage even the more susceptible nerve cells. Naturally acquired immunity may rest largely on exposure to such low titer strains, which may be quite distinct from those capable of inducing epidemics of the paralytic disease.

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Micro Determination of Serum Proteins by Gasometric Carbon Analysis.

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The successful adaptation of the Van Slyke manometric apparatus to the measurement of carbon in organic compounds by wet combustion is well illustrated by the micro-gasometric measurement of serum lipids.[1] The small quantity of material needed for analysis, the precision afforded by manometric technic, rapidity and ease in preparation of substances for lipid carbon analysis, led us to attempt the estimation of serum proteins by a technic designed to measure the protein carbon rather than the protein nitrogen which forms the basis for quantitative methods heretofore proposed.

There are several objections to the use of nitrogen data alone for the quantitative expression of protein. First, the commonly accepted use of the factor 6.25 x protein nitrogen = grams of protein takes for granted a constancy in the amino-acid structure of proteins which we have no valid right to assume, particularly in the analysis of abnormal changes in serum proteins occurring in pathological conditions. Diamino carboxyllic acids and certain of the hetero-cyclic amino-acids, for instance, contain on an average twice as much nitrogen as mono-amino-acids, yet no cognizance is taken of this fact or of the posibility of change in ratio of certain of these amino-acids in pathological states when nitrogen alone is used as the element to which the empirical factor 6.25 is applied for protein conversion. The objection that the use of carbon is also an empirical approximation is met by the fact that while the nitrogen may be increased by as much as 100%, the elementary carbon in di-amino-acids is approximately the same as in the mono-amino group. When multiplied by the nitrogen conversion factor, moreover, such differences in amino nitrogen content may conceivably produce errors of considerable magnitude.

Another argument in favor of carbon as the reference element in the determination of protein is the fact that it forms relatively a much greater percentage of the total protein components than does nitrogen, hence a small error in analytical data is kept relatively less due to the smaller conversion factor.

Another legitimate objection to nitrogen analyses is the well-known difficulty encountered in digestion. The relative ease and simplicity with which carbon combustions may be made obviate many of the difficulties inherent in protein digestion technics preparatory to nitrogen analyses. Moreover, no added determination and correction for non-protein nitrogen is necessary. Last, but perhaps first in importance, the accurate carbon analyses possible on comparatively minute amounts of materials make the carbon combustion technic for protein determination applicable to relatively small amounts of serum (0.1 cc) such as may be obtained readily from finger blood and from small experimental animals.

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[1] Van Slyke, D. D., Page, I. H., and Kirk, E., *J. Biol. Chem.*, 1933, **102**, 635.

Technic. Except for the initial dilution, the procedure for a complete analysis can be carried out in one combustion tube, thus obviating the chance of error through losses of material in transference. As many as 20 or more samples may be prepared for analysis at the same time, the limitation being only convenience in handling, centrifuging, and storing of materials preparatory to combustion.

Fractionation is carried out by the Campbell and Hanna[2] method, the filtrate saved for tungstic acid precipitation of remaining protein. The choice of tungstic acid as the protein precipitating agent seemed advisable for several reasons. Folin and Wu[3] early called attention to the fact that non-protein materials were not carried down to any significant degree with tungstic acid, hence the wide adoption of tungstic acid filtrates for differential analyses of non-protein constituents of blood. Moreover, tungstic acid produces a heavy protein aggregation which settles promptly under centrifugation and remains tightly packed through the procedure of capillary suction and removal of supernatant liquid.

Combustion technic and apparatus has been described in detail by Van Slyke, Page and Kirk[1] so that description need not be repeated here. The method of combustion, once fractionation and precipitation have been carried out follows without deviation that proposed by these authors for lipid carbon determination.

Reagents. (All materials are Reagent Quality).

1. Tungstic acid precipitation reagent. Prepared according to the method of Folin, and modified by Haden.[4]
2. Alcohol-ether mixture, 3:1.
3. Sodium sulfite 21%, for use in globulin separation as proposed by Campbell and Hanna.[2]
4. Chromic acid digestion mixture, prepared according to Van Slyke, Page and Kirk.[1]

Procedure. Total Protein Carbon: 0.1 cc of serum is measured carefully into a 10 cc volumetric flask, rinsed from pipette and made to volume with physiological saline. Invert several times to insure thorough mixing. One cc aliquots are measured into pyrex combustion tubes* and the protein precipitated by the addition of 5 cc of tungstic acid reagent. Allow to stand 2-3 minutes for aggregation to occur. Centrifuge 10 minutes at 2000 R.P.M., after which time the

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[2] Campbell, W. R., and Hanna, M. I., *J. Biol. Chem.*, 1937, **119**, 15.

[3] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81.

[4] Haden, R. L., *J. Biol. Chem.*, 1923, **56**, 469.

* Combustion apparatus may be purchased from Eimer and Amend, New York, Catalogue No. 18237.

protein will be packed tightly at the bottom of the combustion tube. Remove clear supernatant liquid by a capillary tube attached to suction. The precipitate is then washed twice with 5 cc portions of tungstic acid reagent, centrifuging and removing supernatant as above. Finally the precipitate is washed once with 5 cc portions of alcohol-ether mixture to remove any trace of serum lipids which may have been carried down with the precipitate, the supernatant removed with capillary suction. The precipitate is then heated on water bath to drive off traces of alcohol and ether, and set aside under cover for combustion.

Globulin Fractionation: 2.4 cc of 21% sodium sulfite is added to 0.1 cc of serum, the two are mixed several times by gentle inversion and allowed to stand 10 minutes for globulin precipitation to occur. Mixture is then poured twice through the same filter (No. 40 Whatman's) and 0.5 cc aliquots measured into pyrex combustion tubes as described above. The protein is precipitated by 5 cc of tungstic acid reagent plus 2 drops of 10 normal sulfuric acid to neutralize the sodium sulfite present. Allow material to stand for 5 minutes, centrifuge and wash in manner described above for total protein.

Combustion is carried out after the method for organic carbon described by Van Slyke, Page and Kirk,[1] and manometric values converted to milligrams of carbon according to factors given by these authors.

In order to express protein carbon data in terms which could be compared critically with data derived from protein nitrogen determination it became necessary to make simultaneous determination of protein nitrogen with protein carbon. Protein nitrogen was determined by gasometric micro-kjeldahl technic according to the method of Van Slyke.[5] In a series of normal pooled sera, the ratio of carbon to nitrogen was found to be a constant (Table I). This ratio for normal serum protein agrees favorably with previously reported values from elementary analyses.[6]

TABLE I.
Carbon and Nitrogen Analyses of Pooled Serum Protein.

Pooled Sample No. *	Carbon %	Nitrogen %	C/N	N Factor	N Factor / C/N Ratio
A	35.60	10.46	3.40	6.25	1.84
B	34.57	10.09	3.42	6.25	1.83
C	30.05	8.86	3.395	6.25	1.84

*Each pooled sample contained 10 normal sera.

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[5] Van Slyke, D. D., *J. Biol. Chem.*, 1926, **71**, 235.

[6] *Handbuch der Biochemie des Menschen und der Tiere*, 1909, pp. 280-285.

TABLE II.
Total Protein by Carbon Analysis.

Sample No.	Mg Carbon/cc	Carbon Factor	Total Protein, g %
1	.300	1.84	5.52
	.301	1.84	5.53
2	.3645	1.84	6.71
	.364	1.84	6.70
3	.3555	1.84	6.54
	.356	1.84	6.55
4	.379	1.84	6.98
	.378	1.84	6.96

Albumin and Globulin by Carbon Analysis

Sample No.	Total Carbon mg/cc	Albumin Carbon mg/cc	g %		
			Total Prot.	Albumin	Globulin*
1	.300	.1775	5.52	3.26	2.26
	.301	.1765	5.53	3.25	2.28
2	.314	.1875	5.78	3.45	2.33
	.317	.1865	5.83	3.43	2.40
3	.348	.2010	6.41	3.70	2.71
	.349	.2000	6.43	3.68	2.75
4	.361	.2102	6.64	3.87	2.77
	.3615	.2110	6.65	3.88	2.77

*By difference.

In Table II are listed duplicate pairs of total protein and globulin-by-difference values, indicating the degree of consistency possible by the protein carbon technic. In all instances the second decimal place is significant.

The carbon ratios given above represent determinations on normal sera. An investigation into the nitrogen and carbon content of serum proteins occurring in various pathological conditions is under way. Also an attempt is being made to apply carbon combustion technic to the determination of other serum and plasma constituents, such as fibrinogen, euglobulin, pseudoglobulin, etc. It is felt that carbon analyses, in addition to nitrogen studies, may help to characterize certain pathological proteins more closely than is now possible with nitrogen analyses alone.

Summary. 1. A manometric micro-method for the determination of serum proteins by protein carbon estimation has been outlined which gives consistently reproducible results on as little as 0.1 cc of serum. 2. Certain advantages, both technical and theoretical, in the use of carbon rather than nitrogen as the reference element in serum protein determination is discussed.