During the course of this work Mills, Briggs, Elvehjem and Hart[5] reported that a concentrate from liver representing Peterson's "eluate factor"[6] was potent as a growth factor in chicks and that it also prevented the development of anemia (low hemoglobin). On the basis of the above observations these authors suggested that Hogan's antianemia factor and Peterson's "eluate factor" might be identical. They also pointed out the similarity between the two factors with respect to their alcohol insolubility and adsorbability on Fuller's earth at acid pH levels. Following the appearance of this publication, we assayed our concentrates of the antianemia factor by the microbiological growth method and found them to be highly active in growth factor activity for *Lactobacillus* ε (Peterson's "eluate factor"). The repeatedly recrystallized vitamin produces approximately half-maximum growth of *Lactobacillus casei* ε in a concentration of 0.00005γ per cc of culture media. These observations demonstrate conclusively the identity of Hogan's antianemia factor and Peterson's "eluate factor."

Likewise, during the course of our isolation work on the antianemia factor, Mitchell, Snell and Williams[7] reported the preparation of a concentrate from spinach which was very active in stimulating the growth of *Streptococcus lactis R.* or *Lactobacillus casei* ε in comparable dosage. They expressed the opinion that they had "what appears to be a nearly pure chemical entity." They stated that it contained nitrogen, no sulfur or phosphorus and had a molecular weight of 500 as determined by diffusion methods and suggested the name *folic acid* for this microbiological growth factor. Peterson[4] has discussed the probable identity of his "eluate factor" with the "folic acid" factor of Williams. It appears probable that the chick antianemia factor, Peterson's "eluate factor" and Williams' "folic acid" factor are the same. We shall discuss later the chemical identity of the chick antianemia factor from animal with that from plant sources.

Since Hogan and his collaborators discovered the vitamin nature of the chick antianemia factor in liver[1,2] and applied the convenient designation vitamin Bc, we propose the retention of this term for the pure crystalline compound from liver until such time as chemical knowledge of the substance may suggest a more suitable name.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

QUANTITATIVE MICRO-ESTIMATION OF ANTIBODIES IN THE SERA OF MAN AND OTHER ANIMALS[1]

QUANTITATIVE micro-methods for the determination of antibody nitrogen, conforming to the rigid criteria of analytical chemistry and involving the use of the micro-Kjeldahl or Teorell procedures have been available for some years.[2,3,4] These methods reach their greatest degree of accuracy with quantities of antibody nitrogen ranging from 0.1 to 1 mg. There is need, however, for a procedure which could be carried out with one fifth to one tenth these amounts, particularly in the case of human sera, in which the antibody content in health, immunity or disease is not likely to be large. The present method, developed to meet this need, has been in use in this laboratory for more than a year and has consistently yielded reproducible results. The principal departures in technique from the earlier method are precautions to ensure sterility during the relatively long period before the precipitates are washed, and colorimetric estimation of the nitrogen. Depending upon the results of preliminary tests three 1 to 4 ml portions of serum are used.

Use of the Folin-Wu-Ciocalteau phenol reagent for the estimation of proteins was advocated by Wu,[5] developed by Anson,[6] rendered more sensitive by Herriott[7] through the addition of minute amounts of copper ion, and further adapted to micro-analysis by

[5] R. C. Mills, G. M. Briggs, Jr., C. A. Elvehjem and E. B. Hart, *Proc. Soc. Exp. Biol. and Med.*, 49: 186, 1942.

[6] B. L. Hutchings, N. Bohonos and W. H. Peterson, *Jour. Biol. Chem.*, 141: 521, 1941; E. E. Snell and W. H. Peterson, *Jour. Bact.*, 39: 273, 1940.

[7] H. K. Mitchell, E. E. Snell and R. J. Williams, *Jour. Am. Chem. Soc.*, 63: 2284, 1941.

[1] From the Department of Medicine, Columbia University, College of Physicians and Surgeons, and the Presbyterian and Babies Hospitals, New York City. Aided in part by a grant from the Commonwealth Fund.

[2] M. Heidelberger and F. E. Kendall, *Jour. Exp. Med.*, 50: 809, 1929; M. Heidelberger, R. H. P. Sia and F. E. Kendall, *Jour. Exp. Med.*, 52: 477, 1930.

[3] M. Heidelberger, F. E. Kendall and C. M. Soo Hoo, *Jour. Exp. Med.*, 58: 137, 1933; M. Heidelberger and F. E. Kendall, *Jour. Exp. Med.*, 61: 559, 1935; M. Heidelberger and E. A. Kabat, *Jour. Exp. Med.*, 60: 643, 1934.

[4] M. Heidelberger, F. E. Kendall and T. Teorell, *Jour. Exp. Med.*, 63: 819, 1936.

[5] H. Wu, *Jour. Biol. Chem.*, 51: 33, 1921.

[6] M. L. Anson, *Jour. Gen. Physiol.*, 22: 79, 1938–39.

[7] R. M. Herriott, *Proc. Soc. Exp. Biol. Med.*, 46: 642, 1941.

Altschul.[8] Washed specific precipitates containing as little as 1 γ of N yield a definite blue color by the modified method and this may be read in a photoelectric colorimeter or a spectrophotometer[9] at λ = 650.

Since heat-inactivation may damage antibody in weak antisera,[10] complement, which may add nitrogen to certain specific precipitates,[11] is removed by addition of an antigen and the corresponding rabbit antibody unrelated to the immune system to be analyzed. These may be added either separately or in the form of finely divided specific precipitate suspended in saline. For analyses of antibodies to pneumococci of many types and influenza bacilli, egg albumin (Ea) and rabbit anti-Ea are used in this preliminary step. After centrifugation of the Ea-anti Ea precipitate the supernatant is divided into three equal portions, one of which serves as a blank. To the other two a slight excess of specific polysaccharide (usually 0.005 to 0.02 mg, as indicated by the preliminary tests) is added and the serum and solution are thoroughly mixed. Conical centrifuge tubes of about 8 ml capacity are convenient to use. After one half to one hour at 37° C the tubes are placed in the refrigerator for a week or ten days. Centrifugation and washing in the cold are carried out as in previous papers.[3, 4] The blanks and precipitates are then taken up in water, treated with 0.2 to 0.3 m of 0.1 *N* NaOH until the precipitates are dissolved, and made up to 2.5 ml or more, depending upon the amount of precipitate. Aliquots of 2.0 ml are mixed with 6 ml of clear 12.5 per cent. Na_2CO_3 solution and allowed to stand for 1 hour to ensure maximum color development later. 1 ml of Folin reagent freshly diluted with two parts of water is then added. After 20 to 30 minutes the duplicates may either be read directly against the blank, with the latter set at 0 optical density or 100 per cent. transmission, or all tubes are read against a blank of 2 ml of water to which the above reagents have been added. Optical densities are read and converted into antibody N by means of a factor obtained with known amounts of antibody N. The optical density is proportional to the amount of antibody N, but may differ for antibodies of the different animal species and may not be the same as that of normal globulin. Indicated differences in the color values will be studied in greater detail. Table I gives representative analyses carried out by earlier modifications of the method differing in detail.

TABLE I

Species, Serum Volume	Specific polysaccharide and amount used		Optical density*	Factor for conversion to N	Factor for calculation of aliquot to standard vol.	Antibody N per 4 ml serum
ml		*mg*				*mg*
Man, 4.5	Pneumococcus "C,"	0.02	0.320	0.0642	1.445	0.030
" "	" III,†	0.01	0.118	0.0642	1.2	0.008
Horse, 0.5	" "C,"	0.015	0.497	0.0746	10	0.371
Rabbit, 1 (1:10)	*H. influenzae,* B,	0.007	0.522	0.0698	66.8	2.44

* Negative logarithm of transmittance.
† After preliminary absorption with "C" substance.

A more detailed account of the method, its possibilities and its limitations is in preparation.

SUMMARY

A micro-method is described by which as little as 10γ of specific precipitate nitrogen may be determined with a fair degree of accuracy. The error in repeated determinations is about ± 2γ.

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[8] A. M. Altschul, personal communication.

[9] A Coleman Universal spectrophotometer was used in these studies.

[10] Unpublished experiments in this laboratory.

[11] (a) M. Heidelberger, *Jour. Exp. Med.*, 73: 681, 1941; (b) M. Heidelberger and M. Mayer, *Jour. Exp. Med.*, 75: 285, 1942.

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