

sponsor the selection of a small group of representative nomenclaturists to "take over," at least for the time being. Full cooperation between the two committees proved impractical without great loss of time, so the Mammalogists' committee, after receiving the approval of the majority of the entomologists, selected and organized the group as indicated above. The membership of the Mammalogists' committee which carried this out was as follows: A. Cabrera, E. R. Hall, G. S. Miller, Jr., W. H. Osgood, T. S. Palmer, G. G. Simpson and G. H. Tate.

The "Council" thus formed is frankly experimental and the method of its genesis is unusual, but those responsible for it have felt that any other method, especially in these times when large general meetings are interdicted, would defeat itself in prolonged discussion and fatal delay. Under restrictions which the Council has placed upon itself it is hoped that it may be a wholly representative and cooperative body. A brief set of by-laws has been adopted in which important provisions are to the effect (1) that the active membership shall not exceed fifteen, (2) that addi-

tions and replacements shall be made only from candidates nominated by large associations of zoologists, and (3) that no important action can be taken without first obtaining the opinion of at least two nomenclatural committees of national societies and several individuals not members of the Council. A judicial function is thus implied preceding any legislation.

It does not propose to supersede any existing special nomenclatural committees, but on the contrary it is designed to stimulate them to greater interest and more action. Its post-war status is problematical, but at least as a temporary measure and an effort in a direction urgently needing attention, it is hoped it may find support. At an early date it is proposed to increase the membership from nine to eleven in order to cover important branches of zoology which are not now well represented.

The by-laws giving details of proposed action will soon be available for distribution to any interested zoologists who may apply for them.

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Secretary

SPECIAL ARTICLES

ISOLATION OF THE ANTIANEMIA FACTOR (VITAMIN B_c) IN CRYSTALLINE FORM FROM LIVER*

SOMETIME ago Hogan and Parrott¹ pointed out that under certain dietary conditions chicks fail to grow, and develop a severe anemia which can be cured with liver extracts. They recognized the responsible factor as being an unidentified member of the B complex and for convenience designated it vitamin B_c. O'Dell and Hogan² developed an assay procedure with chicks, and succeeded in concentrating the factor in crude liver extract about sixty-fold.

In the fall of 1940 we undertook the isolation of this vitamin. We have recently succeeded in obtaining the compound in pure form. It crystallizes from water in orange-colored spherulites which exhibit typical parallel crossed extinction. After repeated recrystallization it separates in clusters of thin yellow spear-head shaped platelets.³ It does not melt below 360° C. but darkens and chars from about 250° C. Analysis of an ash-free specimen gave the following percentage composition: C 50.50, 50.63; H 4.78, 4.78; N 19.91.

The compound yields a crystalline methyl ester which has no melting point, contains less than 5 per

cent. of the activity of the original acid and can be converted back to the crystalline acid.

When the crystalline acid is administered to day-old chicks on a prophylactic test⁴ at a dosage level of 2.5γ per gram of deficient ration the chicks grow normally and at the end of 4 weeks exhibit no anemia. Studies under way will establish the minimum dosage level necessary. The data in Table I summarize the

TABLE I
BIOLOGICAL RESPONSE TO CRYSTALLINE VITAMIN B_c

	No. of chicks	Level per gm of ration	28-day test		
			Hgb. gm 100 cc	Per cent. Hematocrit	Weight (gm)
1. Basal ration (negative controls)	127	...	5.51*	14.2	75.8
2. Broiler ration (positive controls)	18	27.7	169.5
3. Basal ration + crude concentrate	16	0.18 cc	9.90*	29.7	191.1
4. Basal ration + semi-pure fraction	4	5.0γ	32.5	198.5
5. Basal ration + vitamin B _c (crystals)	9	2.5γ	10.95*	30.1	196.3

* Number of chicks tested was 11, 6 and 6, respectively.

results of a preliminary demonstration of its potency on the chick.

⁴ The details of this method will be published elsewhere by Mr. C. J. Campbell, whose valuable assistance on the animal assay work is hereby acknowledged.

* This article was received for publication on April 8, 1943.

¹ A. G. Hogan and E. M. Parrott, *Jour. Biol. Chem.*, 132: 507, 1940; 128: Proc. xvi, 1939.

² B. L. O'Dell and A. G. Hogan. In press.

³ A complete crystallographic description will be given later by Professor C. B. Slawson, of the University of Michigan.

During the course of this work Mills, Briggs, Elvehjem and Hart⁵ 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 0.00005 γ 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 Williams⁷ 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. Peterson⁴ 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 ANIMALS¹

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-Ciocalteu phenol reagent for the estimation of proteins was advocated by Wu,⁵ developed by Anson,⁶ rendered more sensitive by Herriott⁷ 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.