and the book is profusely illustrated by constitutional formulas where structures are sufficiently well established to justify this. The tasks of the compositors and proofreaders in the case of some of the more complicated and extensive of these formulas must have been particularly laborious and difficult.

This masterly treatise, in the wealth of its documented information, its wide sweep and its up-to-dateness, is *facile princeps* among English books in its own chosen field. So far as the reviewer is informed, nothing at all comparable has appeared since the publication twenty-five years ago of Green and Everest's "The Natural Organic Colouring Matters." Between that date and this, the whole great field of the carotenoids, which is not even mentioned in Green and Everest's book, has experienced a marvelous development and elucidation. Carotene has been recognized as the precursor of the all-important vitamin A. The constitution of many of the carotenoids has been determined, as well as their exceedingly interesting chemical and biological relationships.

This carotenoid chapter also will enable the reader to see what has been contributed to this group since the appearance five years ago of a similar chapter in the first edition of Gilman's "Organic Chemistry."

Realms of still more recent exploration, which find a place in this new book, include the pigments of butterflies' wings, investigated by Wieland, Schöpf and their collaborators, and called by them "pterins." So that even the beautiful dazzling colors of butterflies and of certain insects have been unable to escape the insatiable prying curiosity of the chemist, who has proposed structural formulas for many of them.

Another and much more important field, which twenty-five years ago was largely a *terra incognita,* is that of the blood and bile pigments, to which Hans Fischer and his co-workers have made so many and such splendid contributions. The structural relationship of porphin to the phthalocyanine synthetic pigments of Linstead and his associates is a striking fact.

Curcumin, the principal pigment of turmeric, still remains pretty much in a class by itself (Chapter 2), so far as its chemical constitution is concerned.

Chapters 3 and 4 follow approximately the same general lines of chemical classification as Green and Everest, so far as the older natural pigments are concerned, and are enriched by many new compounds and illuminated by the light of numerous fresh investigations.

One of the chief contributing factors in the elucidation of the chemical nature of those natural pigments which are present so often in infinitestimal amounts has been the remarkable development of microchemical methods of all kinds, stemming from Pregl's pioneer work in the microanalytical field.

As noted in its sub-title, this book is concerned only with natural compounds possessed of visible color, *i.e.,* with pigments and not with dyestuffs as such. The tinctorial properties are incidental and not the governing factor in determining the appropriateness of including a colored compound. On the other hand, attention is constantly called to the absorbingly interesting biological relations and implications encountered.

The chemist will find in this book vistas into many new and fascinating worlds, often but little explored, which beckon alluringly and challenge the adventurous.

The book is warmly commended to all organic chemists. It is deeply to be deplored that the senior author could not have lived to see the fruition of his labors.

MARSTON TAYLOR BOGERT

SPECIAL ARTICLES

HUMAN COMPLEMENT[1]

THIS is a summary of a detailed study of human complement, including the structure and functions of the complement components.[2] The method used for the separation of the mid- and end-pieces of guinea pig complement proved to be inadequate for the separation of the corresponding portions of human complement. The method finally adopted consisted of dialysis of fresh human serum against a phosphate buffer of ionic strength 0.02 and pH 5.4. The dialysis is carried out with mechanical rotation at 1° C for 24 hours or longer, depending on the amount of serum employed. The precipitate, which corresponds to the mid-piece, is washed with cold phosphate buffer of ionic strength 0.02 and pH 5.4. If this precipitate is to be stored it is dissolved in a phosphate buffer of ionic strength 0.3 and pH 6.6.

The supernatant, which corresponds to the end-piece, is neutralized by the addition of 0.02 ml of 1N NaOH per ml, and made isotonic with 18 per cent. NaCl solution. The supernatant is best preserved at 1° C in 1:1.2 dilution in isotonic saline or in 1:2.5 dilution in phosphate buffer of pH 6.6 and ionic strength 0.3. The details of this method will be published elsewhere.

The mid-piece and end-piece obtained by this method

[1] Aided by a grant from the Commonwealth Fund.

[2] For earlier studies *cf.* Mackie, T. J.: *J. Immunology,* 1920, 5: 379; Osborn, T. W. B., "Complement or Alexin," London, Oxford Univ. Press, 1937.—Hegedüs, A., and Greiner, H., *Zeitschr. f. Immunitätsf.,* 1938, 92: 1.

are individually inactive but fully active when combined.

The human complement in one ml of human serum can be deprived of its third component by incubation at 37° C for 1 hour with 1.35 mg of the insoluble carbohydrate of yeast ("zymosan") prepared in this laboratory.[3]

The fourth component is inactivated with 0.2 ml of 0.16 M ammonium hydroxide per ml of fresh serum. The mixture is first shaken and incubated for 1 hour at 37° C; neutralization is then carried out by the addition of 0.032 ml of 1 N HCl. The fourth component can also be inactivated with hydrazine (0.1 ml of 0.16 M hydrazine per ml of fresh serum).

Human complement is inactivated at 52° C for 30 minutes by virtue of the destruction of C'2.

In summary, human complement is composed of four components similar to but not entirely identical with the components of guinea pig complement. It was found that the third component (C'3) is the only component which is effectively mutually substitutive in human and guinea pig complement systems. This is the only component that has features suggestive of enzymic nature. Human C'1 has been purified by ammonium sulfate precipitation and phosphate buffer extraction. It is characterized as a euglobulin with an electrophoretic mobility of 2.9×10^{-5} in veronal buffer of pH 7.8 and ionic strength 0.1. This preparation of C'1 comprises 0.8 per cent. of the total serum proteins. A protein fraction comprising 1 per cent. of the total serum proteins and containing nearly all C'2 has been prepared.

Evidence has been obtained to show that the bactericidal action of human serum against *Vibrio comma* is a result of the joint action of complement and antibody; and that this bactericidal effect is destroyed by the inactivation of any one of the four components of complement. The bactericidal action is restored to a specifically inactivated complement by the addition to it of the particular component which it lacks. It has also been found that the bactericidal action of whole human complement against this vibrio can be fortified by the addition of the end-piece ($pH = 5.4 - \mu\ 0.02$ supernate) of human complement.

Fixations of whole human complement and specifically inactivated human complements to viable sensitized *Vibrio comma* have been investigated. From whole human complement nearly all of the C'3 activity is removed during fixation and only small amounts of the activities of the other three components are removed.[4] However, sufficient amounts of the components are fixed so as to cause the destruction of the vibrios. From complement deprived of C'3, the other three components are fixed to the sensitized organisms but bactericidal action is not exerted. However, when C'3 is added to this complex, bactericidal activity occurs.

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PIGMENT PRODUCTION BY SULFONAMIDE-RESISTANT STAPHYLOCOCCI IN THE PRESENCE OF SULFONAMIDES[1]

IN a previous report[2] the *in vitro* and *in vivo* production of sulfonamide-resistant strains of staphylococci was described. During the past year, investigations have been carried out concerning the mechanism of this resistance. Ten additional resistant strains of staphylococci have been shown to produce a substance which inhibits the antistaphylococcic action of the sulfonamides. It has been found that this inhibitory substance has several of the properties of p-aminobenzoic acid (PAB). These observations confirm the report of Landy and his associates,[3] who studied two sulfonamide-resistant strains of staphylococci supplied by us, and with a microbiological assay concluded that the sulfonamide inhibitor produced by these strains was PAB.

A standard test has been utilized for determining the *in vitro* resistance of staphylococci to the sulfonamides. The organisms were grown for several generations in a water-clear synthetic medium.[4] One tenth cc of a 10^{-3} dilution of a 24-hour culture was added to a series of tubes containing 10 cc of the medium with varying concentrations of sodium sulfathiazole. The contents were incubated for 48 hours at 37 degrees C, and bacterial growth ascertained and expressed in terms of turbidity. The growth of non-resistant strains of staphylococci was completely inhibited by less than 1 mg per 100 cc of sodium sulfathiazole; whereas, a concentration of 200 mgs or more was necessary for the sulfonamide-resistant strains.

While the foregoing studies on sulfonamide-resistance were in progress, an interesting and reproducible phenomenon was observed. Control cultures of either non-resistant or sulfonamide-resistant strains of staphylococci in synthetic medium attained their

[1] Aided by grants from the Charles P. DeLaittre Research Fund and from the Committee on Scientific Research of the American Medical Association.

[2] J. J. Vivino and W. W. Spink, *Proc. Soc. Exp. Biol. and Med.*, 50: 336, 1942.

[3] M. Landy, N. W. Larkum, E. J. Oswald and F. Streightoff, SCIENCE, 97: 265, 1943.

[4] G. P. Gladstone, *Brit. Jour. Exp. Path.*, 18: 322, 1937.

[3] *Jour. Biol. Chem.*, 137: 139. 1941.

[4] Human C' has been found by Heidelberger and Mayer (*Jour. Exp. Med.*, 75: 285, 1943) to add roughly as much N to specific precipitates as does guinea-pig C'.