simple chemical compounds, these products are giants from 30 to 30,000 times as heavy as a molecule of water.

It is the purpose of this book to describe methods by which this chemical transformation or polymerization may be achieved. The description is limited to those products which have achieved commercial importance. Theories of polymer formation (52 pages), condensation polymers (62 pages), vinyl polymers (59 pages), synthetic rubbers (57 pages), resins derived from natural products, particularly rubber and cellulose (37 pages), and application of synthetic resins (24 pages), are adequately treated. The author uses the pragmatic approach and makes a distinction between polymers formed by condensation and those secured by polymerization. This method is useful and adds to the ready presentation of those products where both types of reaction mechanisms are used to bring about an increase in the molecular weight. To each chapter is appended a series of review questions and a good selection of special and, where possible, general references.

The book is recommended to any one who desires a concise summary of the chemistry of synthetic resins and rubbers.

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SPECIAL ARTICLES

AGGREGATION IN SOLUTION OF A SYNTHETIC HAPTEN

A SYNTHETIC hapten which precipitated specifically with antibody was first produced by Landsteiner and van der Scheer.[1] Later Marrack[2] pointed out that if a theory of serological reactions which we may refer to as the "alternation" (or "framework") hypothesis[3] is correct, any compound containing as many as two specific groups capable of combining with antibody should be able to form a precipitate; and this prediction was later tested by Hooker and Boyd,[4] by Pauling and collaborators[5] and by Boyd.[6] In some of these experiments precipitation of hapten by antibody was observed, and Pauling has stated that he considers that these "phenomena provide strong support for the framework theory of serological precipitates. . . ."

It should be recalled, however, that Landsteiner[7] considered that the precipitability of the haptens studied by him was due to their being aggregated in solution, and Hooker and Boyd[4] pointed out that if the haptens studied by Pauling were aggregated under the conditions of the tests this would considerably weaken the support given the theory by this evidence. They further reported observing differences in diffusibility into gelatin of precipitable and non-precipitable haptens, which did in fact suggest that the precipitable ones were aggregated.

We have attempted to study the question of the degree of aggregation in solution of such precipitable haptens, and wish to report here observations made on an arsanilic-phloroglucinol derivative, designated as "VII" in Pauling's earlier paper and in the paper by Boyd[6] and as "XI" in Pauling's later papers. To estimate the degree of aggregation we made measurements of the diffusion coefficient, using the sintered glass disk technic as employed by Northrop and Anson,[8] McBain and others,[9] Lehner and Smith,[10] and Mehl and Schmidt.[11] The disks used were calibrated with both KCl and sucrose.

For hapten "VII" ("XI") we have found a diffusion coefficient at 25° of about 0.109 cm²/day, which would indicate, if the particles are spherical, a particle size in the neighborhood of 12,500. Since the formula weight is 1,122, this would indicate that the hapten is aggregated in solution to the extent of about 10–12 molecules per particle. Details will be published elsewhere.

If other precipitable haptens are aggregated in solution, as we suspect at least some of them are, it is evident that the fact of their precipitabiliy is no stronger evidence for the "alternation" theory than is the precipitation of any antigen, since it seems likely that each particle of the aggregate will have quite a number of accessible specific reacting groups, just as ordinary antigens do. It is evident therefore that we shall in that case have to reexamine most of the conclusions which have been drawn by Pauling and others from experiments with such substances. In particular Pauling's calculations of antibody valence become doubtful.

[1] *Proc. Soc. Exp. Biol. and Med.*, 29: 747, 1932; *Jour. Exp. Med.*, 56: 399, 1932.

[2] "The Chemistry of Antigens and Antibodies," H.M. Stationery Office, London, 1938.

[3] S. B. Hooker and Wm. C. Boyd, *Jour. Immunol.*, 42: 419, 1941.

[4] *Arch. Path.*, 28: 754, 1939; *Jour. Immunol.*, 42: 419, 1941.

[5] *Proc. Nat. Acad. Sci.*, 27: 125, 1941; *Jour. Am. Chem. Soc.*, 64: 2994, 1942.

[6] *Jour. Exp. Med.*, 75: 407, 1942.

[7] "The Specificity of Serological Reactions," Springfield, 1936.

[8] *Jour. Gen. Physiol.*, 12: 543, 1928–29.

[9] *Jour. Am. Chem. Soc.*, 53: 59, 1931.

[10] *Jour. Am. Chem. Soc.*, 57: 497, 1935.

[11] *Univ. of Calif. Pub. in Physiol.*, 8: 165, 1937.

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RAW HEN EGG WHITE AND THE ROLE OF IRON IN GROWTH INHIBITION OF SHIGELLA DYSENTERIAE, STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI AND SACCHAROMYCES CEREVISIAE[1]

RAW hen egg white can cause an inhibition of growth of *Shigella dysenteriae* and other microorganisms which is independent of the avidin-biotin phenomenon. Of ten vitamin factors and thirty-one elements tested, iron alone overcame this egg white inhibition.

In the course of work on the stabilization of dysentery bacteriophage during lyophilization, secondary growth of a strain of *S. dysenteriae* failed to appear in the first tube of a d'Herelle titration of a phage sample lyophilized with raw egg white. Titrations run in the absence of phage with varying concentrations of egg white showed that the inhibition of growth depended upon the amount of egg white added to the medium and was not related to phage activity. 0.02 ml of egg white per ml of nutrient broth (0.3 per cent. Lemco meat extract, 1.0 per cent. Bacto peptone, 0.5 per cent. sodium chloride, adjusted to pH 7.2) inhibited the development of $2-20\times10^5$ bacteria for 24 hours at 37° C. Serial dilution of transfers made from the inhibited cultures to fresh egg white-free broth showed that failure to grow was not due to destruction or lysis of the bacterial cells.

Additions of avidin concentrate to the nutrient broth in an amount equal to twice the estimated avidin content of an inhibiting concentration of raw egg white failed to prevent normal growth of *S. dysenteriae*. Conversely, additions of biotin to the egg white-treated broth in an amount estimated to be twice that required to neutralize the avidin content of the contained egg white failed to permit normal growth of the bacteria.

The effect of pH on the inhibition phenomenon by egg white was such that at pH of 5.8 and below, bacterial growth occurred equal to controls without egg white; at pH's between 5.8 and 6.4, partial inhibition was exerted so that as the pH approached 6.4, initiation of growth was increasingly delayed; at pH 6.4 and above, inhibition of growth was complete for at least 48 hours at 37° C.

When raw egg white was dialyzed against distilled water, the dialyzed material retained fully its ability to inhibit the growth of the bacteria while the dialyzate was without effect. Further, heating samples of egg white adjusted to pH 7.3 with phosphate buffer in nutrient broth for one hour at 40° C., 50° C., 60° C. and 70° C. showed that the inhibiting capacity of the raw egg white is stable at temperatures up to 60° C. but destroyed at 70° C. under the conditions employed.

Corn steep liquor, meat extract and yeast extract, when added in relatively large amounts to nutrient broth in the presence of egg white, permitted growth of *S. dysenteriae* to occur equal to controls without egg white. The following known growth factors were tried singly and in combination: thiamin hydrochloride, riboflavin, nicotinamide, inositol, calcium pantothenate, pyridoxine, biotin, para aminobenzoic acid, choline chloride and tryptophane. All were ineffective in overcoming the egg white inhibition.

When yeast extract was ashed, however, the ash after solution in hydrochloric acid was active. Of 31 elements tested, iron alone overcame the egg white inhibition of growth. Soluble ferrous and ferric salts were effective when used in an amount of approximately 20 gammas of iron for every ml of raw egg white, the ferrous iron being consistently somewhat more active than the ferric form. The amount of "free" iron (dipyridyl method) in the 4.5 ml of nutrient broth used routinely was 0.8–1.0 gammas. Since one ml of egg white requires 20 gammas of added iron, 0.04 to 0.05 ml might be expected to make the "free" iron of the broth unavailable to the bacteria for growth. In general, the results obtained justify this expectation.

Ferrous and ferric ammonium sulfate were added to egg white at various pH's and dialyzed overnight at room temperature against buffered saline. Qualitatively, the data obtained showed that at pH 7.0 and above egg white made both forms of iron undialyzable; at pH 6.0 and to a greater extent at pH 5.0, some ferrous iron could be dialyzed, while ferric iron was undialyzable at both pH's. All results were compared to controls of iron alone in buffered saline at the same pH's. The effect of pH on the dialyzability of iron from egg white-iron salt mixtures finds some parallel in the relation of pH to the inhibition of growth of *S. dysenteriae* in egg white-treated nutrient broth.

When ferric or ferrous iron in concentrations employed in this study were added to raw egg white, a tan to brownish coloration appeared. The depth of color varied directly with the amount of iron added. Possible correlation of color production with the biological phenomenon reported here is being investigated.

Preliminary study of the inhibitive effect of raw egg white on the growth of a strain of *Staphylococcus aureus, Escherichia coli* and *Saccharomyces cerevisiae*

[1] A preliminary report.