Although all these 8 fractions soluble at pH 5.0 could agglutinate Type I pneumococci, they differed not only quantitatively but qualitatively in their reactivity with the homologous polysaccharide and its hydrolytic products. The results of qualitative agglutination and precipitation of these fractions (Table II) reveal the following facts: First, all 8 fractions contain antibodies, for they agglutinate Type I pneumococci and protect mice from an otherwise lethal dose of the organism. Secondly, fraction 8 corresponds to the antibody[2] obtained by the agglutination of Type I antipneumococcal rabbit serum previously absorbed with the homologous polysaccharide. It possesses both agglutinative and protective power but fails to react with the homologous polysaccharide. Thirdly, hydrolysis of the original polysaccharide by acid or both acid and alkali destroys the reactivity with certain fractions of the antibody. These fractions (*e. g.*, Nos. 5 and 6) obtained by the addition of ammonium sulfate gave a heavy precipitate with the original polysaccharide, but only a slight precipitate with the product hydrolyzed by acid and practically none with the product hydrolyzed by both acid and alkali.

The extensive study on the fractionation of normal serum-globulin with salts by Sørenson[3] and other workers supports the current view that it is an easily dissociable complex consisting of the so-called euglobulin and pseudoglobulin in varying proportions. Numerous studies on the distribution of antibodies in antipneumococcal sera have been made by fractionation with ammonium sulfate. However, the estimation of antibody in each fraction is usually made by mouse-protective tests[4] or by the precipitative reaction[5] on the assumption that the same antibody is present in each fraction. This has been found not to be the case in the present study.

9610 P

A 2,4-Dinitrophenylhydrazine Derivative of Dehydroascorbic Acid and the Estimation of Vitamin C.

JOSEPH H. ROE.

From the Department of Biochemistry, School of Medicine, George Washington University, Washington, D. C.

When dehydroascorbic acid is treated with a saturated solution

2 Sørensen, S. P. L., *Compt. rend. trav. lab.*, Carlsberg, 1923-25, **15**, No. 11.

3 Chow, B. F., and Wu, H., *Chinese J. Physiol.*, 1937, **11**, 163.

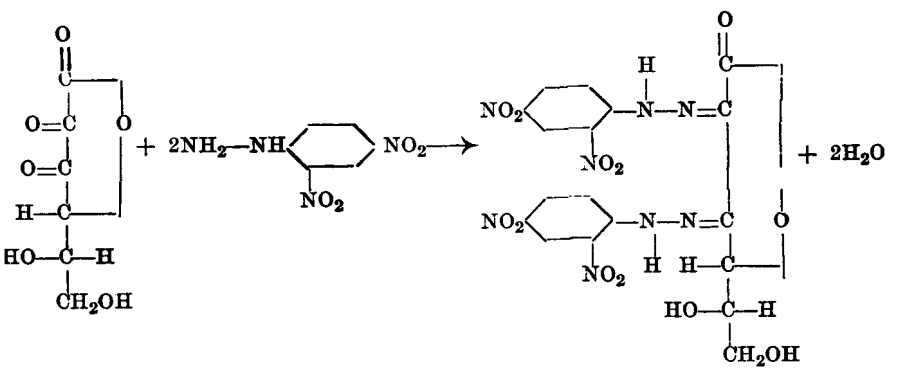
4 Felton, L. F., and Kaufmann, G. J., *Immunology*, 1933, **25**, 165.

5 Chow, B. F., PROC. SOC. EXP. BIOL. AND MED., 1937, **34**, 651.

of 2,4-dinitrophenylhydrazine in N HCl, a reddish crystalline derivative is formed. The reaction is catalyzed by acetic acid. Analysis of the product obtained showed it contains two 2,4-dinitrophenylhydrazine groups in its molecule. The derivative is apparently an osazone of dehydroascorbic acid with the hydrazine groups attached to carbon atoms 2 and 3. The compound melted at 257-259° (corrected) and microanalysis showed it has the following composition: $C_{18}H_{14}O_{12}N_8$

Calculated: C 40.43, H 2.64, N 20.98.
Found: " 40.40, " 2.88, " 21.11.

The evidence indicates the reaction is as follows:



This compound was prepared as follows: 0.2 gm. of pure ascorbic acid in 200 cc. of 10% acetic acid was shaken with norit and filtered. Two volumes of 2,4-dinitrophenylhydrazine in N HCl were added. The mixture was allowed to stand at room temperature for 72 hours and then centrifuged. The precipitate was washed 6 times with distilled water.

Advantage was taken of this reaction to make it the basis of a specific method for the determination of vitamin C. The dehydroascorbic acid osazone is reduced to a colorless compound by warming with 12% HCl containing 10% $SnCl_2$ and the compound is hydrolyzed by heating with these reagents in an autoclave at 15 lb. pressure. Under the latter conditions the released dehydroascorbic acid is converted into furfural, which, upon cooling, is determined by the colorimetric aniline acetate method[1] previously published. Use is made of a standard solution of pure ascorbic acid which is treated in the same way as the acid extract of tissue.

Substances most likely to interfere in this method are free pentoses which will give a centrifugable precipitate in concentrations

[1] Roe, J. H., *J. Biol. Chem.*, 1936, **116**, 609.

greater than one mg. per cc. As the vitamin is extracted from tissues by a procedure which usually involves a 10-fold dilution, and greater dilutions may be made, this possible difficulty with tissues containing free pentose is readily overcome. The method appears to be entirely specific for the estimation of vitamin C.

Osazone formation does not occur in the first few hours after mixing 2,4-dinitrophenylhydrazine with ascorbic acid but later crystals appear slowly. This behavior is interpreted as indicating that ascorbic acid does not react with the hydrazine under these conditions, but upon standing it is oxidized to dehydroascorbic acid which reacts to produce the compound described.

This procedure is particularly advantageous for the determination of vitamin C in urine, for which the indophenol and other oxidation-reduction methods lack specificity. Ascorbic acid added to urine is recovered quantitatively by this method.

In preparing this paper the report of Stewart, Scarborough and Drumm[2] came to our attention. These authors have made a 2,4-dinitrophenylhydrazine derivative from the ascorbic acid in urine. As the method of preparation was not stated and no analyses were reported, a comparison of their derivative with ours cannot be made. The melting point reported (269-271 uncorrected) is about 20 degrees higher than that of our product.

The details of our procedure for the estimation of vitamin C will be published later.

9611

Glycerol Toxicity and Hemoglobinuria in Relation to Vitamin C.

CARL PFEIFFER AND I. ARNOVE. (Introduced by E. M. K. Geiling.)

From the Department of Pharmacology, University of Chicago.

In view of the several recent reports[1, 2, 3] of the curing of paroxysmal hemoglobinuria by the administration of ascorbic acid, it was thought that a study of ascorbic acid in relation to experimental hemoglobinuria might be of interest. Of the numerous substances which produce hemoglobinuria it was found that parenterally injected glycerol was the most practical.

2 Stewart, C. P., Scarborough, H., and Drumm, P. J., *Nature*, 1937, **140**, 282.

1 Armentano, L., *Nature*, 1936, **137**, 910.

2 Lotze, H., *Klin. Woch.*, 1936, **15**, 941.

3 Armentano, L., and Beutsath, A., *Klin. Woch.*, 1936, **15**, 1594.