In every case, leaf material prepared in this way is less brittle and cuts much more easily than after the usual treatment (perhaps because it is in alcohol a shorter time), and even when embedded by more or less careless students shows little or no plasmolysis. This method also requires less attention.

The following modification of the method for "dehydrating woody tissue for paraffin embedding" as given in SCIENCE for January 24, 1930, has been used successfully.

After the killing and fixing solution has been washed out, if the material is soft enough to cut easily, it is placed in 10 pts. glycerine + 90 pts. water and left until the water has evaporated. It is then put into equal parts glycerine and butyl alcohol for about 36 hours, followed by pure butyl alcohol for another 36 hours. It is then embedded as described in the article in SCIENCE. Three to six days in the paraffin bath may be necessary to secure thorough embedding.

If tissues are part hard and part soft, they may be softened in C.P. hydrofluoric acid + 95 alcohol, as this method, according to Dr. E. C. Jeffrey, injures delicate tissues less than hydrofluoric acid diluted with water. If tissues are very hard, they may be put into C.P. hydrofluoric acid for three or four weeks, then washed and treated as described.

This method has the advantage of practically avoiding the use of ethyl alcohol, with the result that the material is less brittle and no harder than before dehydration. The longer time in the paraffin bath does not appear to make woody tissues hard and brittle. This method likewise requires less attention.

In dry weather or in dry climates it has been found that the addition of eight or ten parts of glycerine to preservatives such as alcohol-acetic acid-formalin is efficacious in preventing rapid evaporation caused by imperfect cork stoppers.

In cutting wood sections that are likely to curl, if the microtome knife is kept wet with equal parts of glycerine and 95 alcohol, and each section is permitted to remain on the blade a few seconds after cutting and is then transferred to a dish of the same solution, this difficulty is entirely avoided.

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# SPECIAL ARTICLES

## DATA ON A PROTEIN-ANTIBODY SYSTEM

IT was recently shown by the writers that the entire course of the precipitin reaction between Type III pneumococcus specific polysaccharide and purified homologous antibody could be expressed quantitatively by simple equations that follow the mass law.[1] Since it was of interest to determine whether a similar method of treatment might be applied to a true antigen-antibody system, recourse was had to the compound azo proteins, so fruitfully employed in the solution of other immunochemical problems by Landsteiner and by Avery and Goebel. It was felt that if a protein dye could be synthesized of more intense color and more rigorously freed from its component substances than those used by these workers, a possible means would be at hand for distinguishing between antigen and antibody nitrogen both in the precipitates and supernatants, since the antigen could be determined colorimetrically. A purplish-red disazo protein dye, *R-salt-azo-benzidine-azo-egg albumin,* was finally isolated in a form which satisfied these requirements, the more so as the antibody solutions obtained by sodium sulfate fractionation of rabbit antisera to the dye were substantially colorless.

By methods analogous to those used in the first paper[1] it was found that at the lowest concentration of dye protein used, 1: 67000, the mean ratio between antigen and antibody precipitated was 1: 15 (8 determinations); at the equilibrium point,[1] at which the proportions of the reactants were such that both antigen and antibody were present in solution, 1: 7.5 (6 determinations), and in the inhibition zone, after the maximum precipitate was reached, 1: 3 (14 determinations).

These preliminary data indicate that the composition of the solid phases formed by the precipitin reaction between the dye and its antibody may be expressed by the three limiting equations:

$$\mathrm{An} + \mathrm{Ab} \rightleftarrows \mathrm{AnAb},$$
$$\mathrm{AnAb} + \mathrm{An} \rightleftarrows \mathrm{An_2Ab}, \text{ and}$$
$$\mathrm{An_2Ab} + 3\mathrm{An} \rightleftarrows \mathrm{An_5Ab},$$

in which An = antigen and Ab = antibody. The data so far obtained are insufficient for a decision as to whether the soluble compound formed in the inhibition zone is $\mathrm{An_6Ab}$ or $\mathrm{An_7Ab}$. Further work is in progress and a detailed report will be made when this has been carried out.

Whether or not modification of the above ratios be necessary, it would appear that the precipitin reaction between a true antigen and its antibody is essentially the same as the precipitin reaction in a hapten-antibody system or, for that matter, essentially the same as a typical inorganic precipitation reaction, and may

[1] *Jour. Exp. Med.*, 50: 809, 1929.

be quantitatively expressed by suitable application of the laws of classical chemistry.[2]

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## THE AMOUNT OF CIRCULATING PRECIPITIN FOLLOWING THE INJECTION OF A SOLUBLE ANTIGEN

IN the preceding note preliminary data were reported on the precipitin reaction between a protein and its homologous antibody. These data permit for the first time a calculation of some theoretical interest; namely, the amount (weight as opposed to titer) of circulating precipitin in an animal following immunization by a given amount of antigen. For example, rabbits 49 and 54 were injected with small doses of *R-salt-azo-benzidine-azo-egg albumin* until a total of 21.6 mg of the dye had been given. The animals were bled 10 days after the last injection, and antibody solutions were prepared by sodium sulfate fractionation and made up to double the serum volume. This may be considered as blood volume for the present purpose. The maximum specifically precipitable protein[1] in solution 49 was 1.53 mg per cc; in solution 54, 1.25 mg per cc. Taking the weight of the rabbits as 2 kg and their blood volume as 5.5 per cent. of their weight,[2] or 110 cc, the blood of rabbit 49 contained 168 mg of precipitin at the time of bleeding, while that of rabbit 54 contained 138 mg. Calculated as milligrams of circulating precipitin per milligram of antigen injected, the values are 7.8 and 6.4, respectively.

Naturally these figures are inaccurate, since the exact blood volumes of the rabbits were not known. From the theoretical standpoint, however, it is of interest that they are probably low, since losses undoubtedly occurred in the preparation of the antibody solutions. Moreover, these values can only represent a fraction of the total antibody formed, since storage in the cells occurs as a result of sensitization of tissues and organs. It is also not certain that the circulating precipitin is the only circulating antibody. On the other hand it is considered, as in the preceding studies, that antibody is modified globulin, and that the antibody precipitated is not contaminated with nonspecific serum globulins. Evidence on the latter point will be reported later.

According to Svedberg and Sjögren[3] the molecular weight of serum globulin is three times that of egg albumin. If one assumes antibodies to have about the same molecular weight as the globulins with which they are associated, and the egg albumin dye to have about the same molecular weight as egg albumin, each dye molecule would have to split into more than two specifically reactive fragments if it participated in the building up of the antibody molecule. However, Landsteiner has repeatedly shown that the specificity of the azo protein dyes is a function of the dye component, rather than of the protein used. It would therefore be reasonable to expect that if the antigen or any of its fragments participated in the building up of the antibody molecule the antibody would be colored. It is true that the crude antibody solutions obtained by fractionation of the sera of animals immunized to the red protein dye were definitely pink, but the color disappeared almost completely on dialysis.

The preliminary data herein presented therefore tend to favor the view that the antigen itself does not participate in the building up of the antibody complex. Further information on this and related questions is being sought along these lines and it is hoped that more decisive figures will be obtained.[4]

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## CORRELATION OF ANTILLEAN FOSSIL FLORAS

CERTAIN Antillean plant beds have been described by Drs. Hollick,[1] Vaughan and Berry,[2] Hodge,[3] Howe[4] and Maury,[5] and a tentative grouping and correlation is now suggested.

*The Nilssonia bed, Porto Rico.*—South of Cidra, Mr. Hodge found plants in a bog iron ore bed.

[2] This study was carried out under the Harkness Research Fund of the Presbyterian Hospital.

[1] *Jour. Exp. Med.*, 50: 809, 1929.

[2] Meek and Gasser, *Am. Jour. Physiol.*, 47: 302, 1918–19.

[3] *Journ. Am. Chem. Soc.*, 50: 3318, 1928; 52: 2855, 1930.

[4] This study was carried out under the Harkness Research Fund of the Presbyterian Hospital.

[1] "Rio Collazo Plant Beds, Porto Rico," "Scientific Survey of Porto Rico and the Virgin Islands," Vol. 7, pt. 3, 1928; "Siparia Flora, Trinidad," *Bull. N. Y. Bot. Garden*, vol. 12, No. 45, 1924; "Rio Guajataca Flora, Porto Rico," *Jour. N. Y. Bot. Garden*, 27: 223–7, 1926.

[2] "Sánchez Flora, Dominican Republic," "Geological Reconnaissance of the Dominican Republic," p. 165, 1921.

[3] "Algae of Coamo Springs Limestone, Porto Rico," "Scientific Survey of Porto Rico," vol. 1, pt. 2, pp. 153–9, figs. 15, 16 (not 18), pp. 195, 228, 1920.

[4] "Algal Flora, St. Bartholomew, Antigua, Anguilla," Carneg. Inst. Wash., Pub. No. 291, pp. 11–19, 6 plates, 1919.

[5] "Los Quemados Flora, Dominican Republic," *Bull. Amer. Paleontology*, No. 30, p. 19, 1917.