dently of external antigenic stimuli, have a low order of specificity and resemble the antibodies produced by immunization. It is proposed that the properdin fraction of serum contains these normal substances which destroy bacteria, neutralize viruses, and destroy red cells, and that the injection of zymosan stimulates the production of antibodies with receptor configurations similar to those found in the properdin of normal serum. Such a proposal would readily explain the sustained increase in bactericidal activity of the serum due to the presence of small amounts of zymosan, also the ability of zymosan to absorb bactericidal activity due to immunization as well as to properdin.

Kabat and Berg(13) in demonstrating the antigenicity of dextran in man, suggested that "In all probability, other polysaccharides composed of only a single sugar, levans, mannans, etc., will also prove to be antigenic in man —". Zymosan belongs to this group of polysaccharides and it may be possible, by injecting zymosan, to increase the "natural antibodies" in the human species.

Summary. 1. Bactericidal activity against *Escherichia coli* B was increased in rabbits and guinea pigs as a result of injections of zymosan mixed with egg-white and incorporated in Freund adjuvants. 2. These rabbits and guinea pigs responded to intracutaneous injection of zymosan with the Arthus phenomenon. 3. Zymosan absorbed the bactericidal activity without affecting the hemolytic activity of the sera. 4. The possible antigenicity of zymosan is discussed.

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Turbidimetric Determination of Proteins with Sulfosalicylic and Trichloracetic Acids. (22601)

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Folin and Denis(1), using a method proposed earlier for the nephelometric determination of protein in milk and digestion mixtures, introduced the turbidimetric determination of protein in urine with sulfosalicylic acid (SA). Subsequently, this technic was extended to protein determination in spinal fluid by Denis and Ayer(2) and in serum or plasma by Looney and Walsh(3). Mestrazat(4) used trichloracetic acid (TCA) for the turbidimetric determination of protein in spinal fluid.

The biuret reaction has almost completely supplanted turbidimetry for the routine determination of protein in serum in the clinical laboratory, but because of its greater sensitivity the turbidimetric method is still widely used for urine and spinal fluid and most textbooks advise the use of SA.

Although some workers(3,5) have reported observing no difference in the turbidity pro-

duced with albumin or globulins by SA, Plötner(6) claimed that by nephelometry the turbidity produced by SA with albumin was about 4 times that with globulin. Bossak and coworkers(7) found that, by turbidimetry, albumin gives about twice the turbidity given by γ-globulin, whereas much better agreement was found using TCA. They, therefore, recommended the use of TCA for the turbidimetric determination of protein in spinal fluid. The difference in turbidity produced by SA with albumin and globulin was again confirmed nephelometrically by Heepe and coworkers(8). Their results, however, showed a parallel difference with TCA.

Obviously, if the degree of turbidity produced is dependent on the relative concentration of albumin and the various globulins, quantitative results are subject to an uncontrollable, and in most instances unknown, variable. This study compares results obtained using SA, TCA, and a mixture of the 2 acids (SA and TCA) giving identical turbidities with albumin and γ-globulin, on a series of sera with a wide range of A/G ratios.

Materials and methods. Human albumin (Lot No. 940 C2B, Hyland Laboratories, Los Angeles, Calif.) and human γ-globulin (Lot No. 2175-249-B, Poliomyelitis Immune Globulin, Lederle Lab. Division, American Cyanamid Co., New York City) were standardized by Kjeldahl N and in turn were employed for standardizing the biuret test and determining relative turbidities produced by SA, TCA and the SA-TCA mixture. The biuret method was employed as the reference method for total protein since biuret equivalents of the various protein components of serum are identical or very nearly so, thus accounting for the close agreement with results obtained by Kjeldahl N determination(9). Precipitation of globulins for determination of the A/G ratio was carried out by the method of Wolfson and coworkers(10). Sera were diluted 1:600, 1:400 and 1:600 with 0.85% NaCl for use with SA, TCA and SA-TCA, respectively. To 4.0 ml of these dilutions was added 1.0 ml of the acid reagent followed by immediate mixing. The SA and TCA reagents were 12.5% and the SA-TCA reagent was a mixture of 3 volumes of the SA reagent and 4 volumes of the TCA reagent. The turbidities were read between 5 and 10 minutes after addition of reagents and after remixing and allowing any air bubbles thereby produced to rise to the surface, in a Bausch and Lomb Spectronic 20 at 420 mμ, using the square cuvettes (light path of 11.5 mm) and water as a blank. The experiments were carried out at room temperature which was 24 ± 2°C.

Results. Acid Concentration. Final concentrations of 2.5 and 5.0% of TCA, SA and the SA-TCA mixture were compared. Because of the greater tendency towards flocculation of the protein precipitate with the higher acid concentration, lower absorbence (A) readings were obtained in general. It was concluded that the finer suspension produced by the lower acid concentration was preferable. *Time of reading.* Albumin consistently flocculated more quickly than γ-globulin. Measurements made on albumin-γ-globulin mixtures and several sera at 1 minute intervals up to 20 minutes showed fairly consistent readings between 5 and 10 minutes after the addition of the acid. If readings are delayed until gross flocculation occurs it is difficult to obtain proper dispersion, a prerequisite to a correct reading. *Gum ghatti.* Use of gum ghatti as a protective colloid(3) was found to delay but not prevent flocculation. Occasionally, its presence appeared to inhibit the development of turbidity. Since no particular advantage was apparent from its use, and perhaps a disadvantage, it was discarded. *Reproducibility.* The 95% limits at a mean absorbence of 0.370 were found to be ± 5% from the mean. *Relative turbidities of human albumin and γ-globulin.* Standardization curves with solutions of these 2 proteins in 0.85% NaCl, using SA, TCA and the SA-TCA mixture, are shown in Fig. 1. One ml of the acids was added to 4 ml of the protein concentrations indicated. With SA, the turbidity given by albumin was about 2.4 times that given by γ-globulin. With TCA, the globulin gave about 1.2 times the turbidity given by albumin. Since the differences produced by these two acids were in opposite directions, a successful search was made by trial and error for a combination of the acids which gave the same turbidities with both

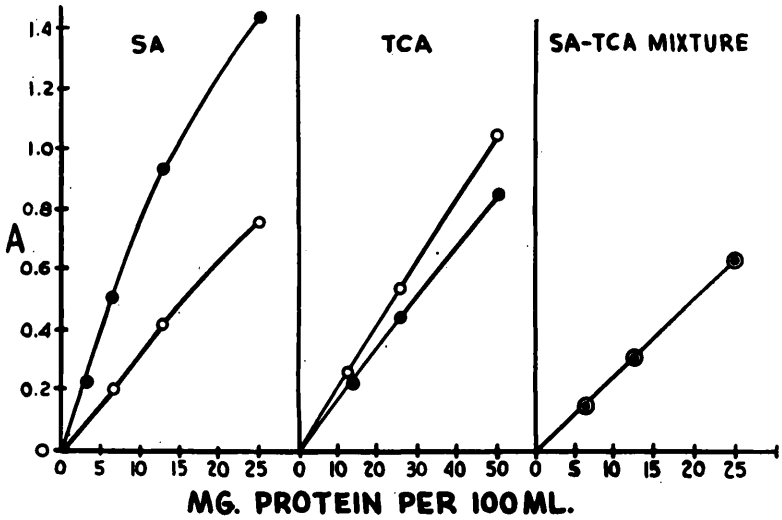

FIG. 1. Standardization curves of human albumin and γ-globulin. ● = albumin; ○ = γ-globulin.

proteins. *Results with sera of varying A/G ratios.* Results obtained with 67 sera are shown in Fig. 2 in which the absorbence values obtained are plotted *vs.* the serum protein concentration. The regression lines shown were calculated as y on x. The correlation coefficients, r, are indicated on the respective graphs. The r values for TCA and the SA-TCA mixture are both significantly greater than that for SA (t test, $P<0.02$). The r value for the SA-TCA mixture, however, is not significantly greater than that of TCA.

To detect the possible dependence of the degree of turbidity produced on the A/G ratio, 100 times absorbence total protein is plotted for the same series of sera *vs.* the A/G ratio in Fig. 3. The regression coefficients, b for y on x, are shown on the respective graphs. The b value for SA is significantly greater than O (F test after analysis of variance of scatter from regression line, $P<0.001$), indicating a dependence of results on the A/G ratio. As predicted from the results with albumin and γ-globulin, the slope is positive. The b values for TCA and the SA-TCA mixture are not significantly different from O, giving no indication of a dependence with these acids on the A/G ratio.

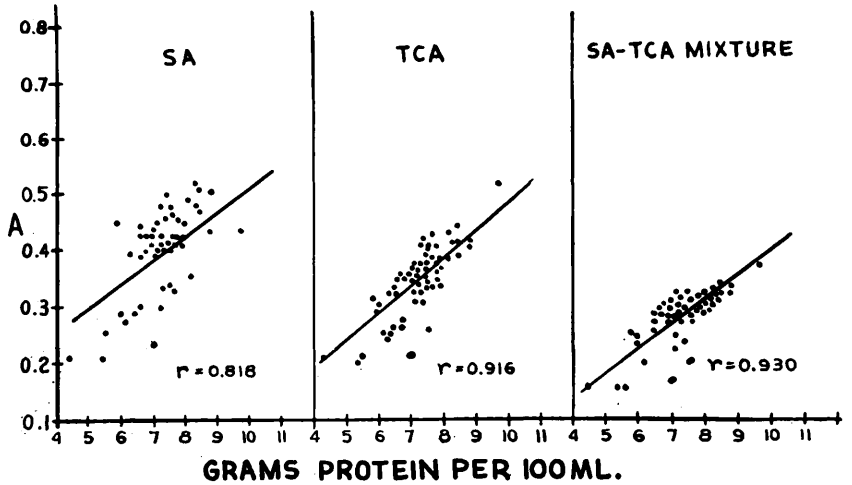

FIG. 2. Scatter diagrams of turbidities produced by 67 sera.

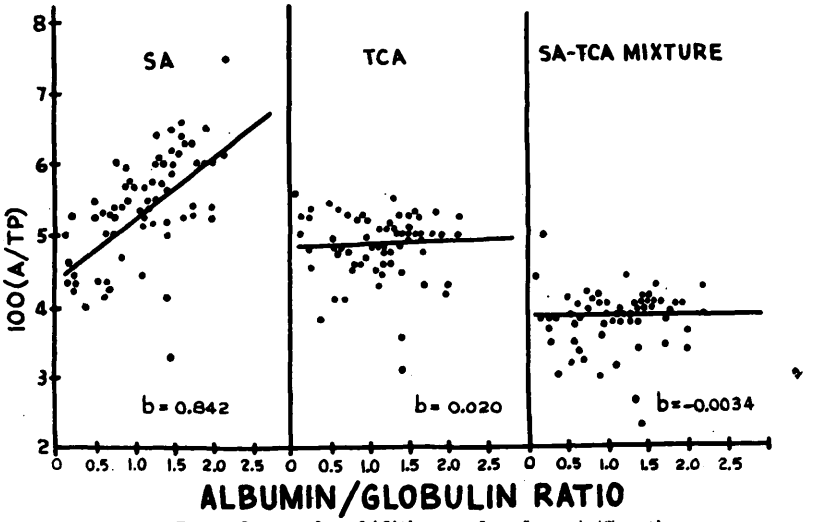

FIG. 3. Dependence of turbidities produced on A/G ratio.

Deviation from the average value of 100 times absorbence, total protein constitutes an estimate of the accuracy of the turbidimetric method. The 95% limits (2 coefficients of variation) for SA, TCA and the SA-TCA mixture are ± 27.2, ± 20.4, and ± 22%, respectively.

Discussion. The estimated accuracy of the turbidimetric method is not very high, being at best about ± 20%. Whether or not this is sufficient for the clinician is beyond the scope of this discussion. The TCA and SA-TCA reagents gave results of significantly greater accuracy than the SA reagent, but improvement was not as great as was expected. There is little doubt that the SA results are correlated with the A/G ratio and thus are subject to an uncontrolled variable. It is quite possible that the turbidities produced by the various proteins other than albumin and γ-globulin present in body fluids are not equal using TCA or the SA-TCA mixture, thus accounting at least partly for the relative inaccuracy observed. Reports by some workers(3,11) of close agreements between results obtained by SA and Kjeldahl N are difficult to understand. Inasmuch as no disadvantage is apparent, but rather a significant advantage, it is believed advisable to supplant SA with TCA for routine turbidimetric determinations. Although a SA-TCA mixture was found which gave better results with albumin and γ-globulin than TCA, results with sera gave no evidence of superiority.

Summary. The turbidity produced by addition of sulfosalicylic acid to a solution of a mixture of proteins is correlated with the A/G ratio. No correlation is apparent when trichloracetic acid is employed. It is suggested, therefore, that trichloracetic acid should supplant sulfosalicylic acid in turbidimetric methods for the determination of protein.

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