the original antibiotic activity can be accounted for. Within the limits of testing procedures, there is no demonstrable union between penicillin and any of the globulins.

To demonstrate further that a union between penicillin and albumin took place, we have attempted to isolate the penicillin-albumin complex by precipitating it in aqueous 50 per cent. alcohol solution at 5° C. The unbound penicillin is soluble in such a concentration of alcohol. The precipitated protein-penicillin complex was re-dissolved with water and re-precipitated with 50 per cent. alcohol without appreciable loss of penicillin activity. To obtain a dried powder of the new compound, the alcohol precipitate was dissolved with water and the solution was lyophylized.

Unlike the sulfonamide-albumin complex, which Davis and others believe is devoid of bacteriostatic property, the penicillin-albumin complex does possess antibiotic activity. If chemical union and not physical adsorption takes place between the antibiotic agent and the albumin, the complex is expected to be more slowly excreted than penicillin itself because of the increase of molecular size. A few preliminary biological studies of this protein have been made. It was found that mice receiving the complex intramuscularly did not excrete the antibiotic agent into the urine as rapidly as mice receiving the same dose of free penicillin by a similar route. The importance of discovering a means to retard the rate of excretion of penicillin has been generally recognized. Partially successful attempts to obtain delayed action of penicillin by use of diodrast[8] or para-amino-hippuric acid[9] have been reported. Likewise, the use of a suspension of penicillin in oil or in oil containing 0.75 to 6.0 per cent. beeswax[10] has been reported to give delayed excretion. However, we are not aware of any report in the literature on a chemical compound of penicillin possessing a delayed excretion rate. It must be stated here that at present we have no evidence that a true chemical compound is formed when penicillin and albumin interact. Since the albumin used is of human origin, the resulting complex might be expected to possess little antigenicity in man. Its metabolism, its immunological properties as well as its stability toward acid, alkali and penicillinase will be reported elsewhere after detailed studies have been completed.

SUMMARY

Penicillin combines with human serum albumin, as demonstrated by dialysis experiments. Unlike the sulfonamide-albumin complex, the penicillin-albumin possesses antibiotic activity. Such a complex can be freed of unbound penicillin by repeated precipitation at 50 per cent. alcohol and obtained as a dried powder. Mouse excretion studies indicated that the penicillin-albumin complex was excreted more slowly than the sodium salt of penicillin.

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[8] C. H. Rammelkamp and S. E. Bradley, *Proc. Soc. Exp. Biol. and Med.*, 53: 29, 1943.

[9] K. H. Beyer, R. Woodward, L. Peters, W. F. Verwey and P. A. Mattis, SCIENCE, 100: 107, 1944.

[10] M. J. Romansky and G. E. Rittman, SCIENCE, 100: 196, 1944.

THE SEROLOGICAL DIAGNOSIS OF SYPHILIS[1]

IN an extensive investigation directed toward the development of a serodiagnostic method for the differentiation between syphilitic and biologic false positive sera, observations have been made which indicate that in the two groups of sera[2] the reactive antibodies differ from each other in certain significant respects. Since previous work has shown that even in strongly reactive syphilitic sera the antibodies constitute but a minute fraction of the total proteins,[3] and that isolation by specific flocculation with lipoidal antigen yields extremely low recovery of the purified product,[4, 5] a practical approach to the problem has been based on non-specific methods of characterization and fractionation of the sera, as described below:

(1) *Electrophoresis.* Electrophoretic analyses were made on 13 normal, 25 syphilitic and 45 biologic false positive sera. The sera from patients with syphilis[6] differed markedly from normal sera in exhibiting decreased albumin and increased *gamma* globulin con-

[1] The work described in this paper was done under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Duke University.

[2] A total of about 300 syphilitic and biologic false positive sera has been used for the investigation of the various phenomena described in this report. Sera accepted as syphilitic were those for which there was unequivocal clinical evidence for infection, whereas the group of allegedly false positive sera may have included some genuinely syphilitic specimens. However, among this group the occurrence of syphilis was fairly well excluded in those cases where the positive serological reaction could be shown to be of transitory nature, and accompanied by such etiologically unrelated diseases as malaria, virus pneumonia, mumps, smallpox vaccination, etc. Serum specimens have been obtained from various Army General Hospitals through Brigadier General Hugh Morgan, M.C., and Major Charles R. Rein, M.C.; from private hospitals through Drs. J. E. Moore and Paul Rosahn; from the Rapid Treatment Center, U. S. Public Health Service, Durham, N. C., through Surgeon Samuel Fisher, and from the clinics of Duke Hospital through Dr. J. L. Callaway.

[3] H. Eagle, "Laboratory Diagnosis of Syphilis," p. 168. St. Louis: C. V. Mosby Company, 1937.

[4] E. Witebsky, *Zent. f. Bakt.*, 122: 70, 1931; *Z. f. Immunf.*, 80: 323, 1933.

[5] O. Bier and E. Trapp, *Jour. Immunol.*, 40: 465, 1941.

[6] These particular sera were obtained from patients who had received therapy of one kind or another.

tents. The difference was in both relative and absolute amounts. Biologic false positive sera from presumably normal individuals showed differences from normal sera which were qualitatively similar to those of syphilitic sera but of considerably smaller magnitude. However, biologic false positive sera from individuals known to have diseases other than syphilis gave findings essentially like those obtained with syphilitic sera. No unique components and no significant mobility differences from normal sera were seen among the serum protein constituents of either the syphilitic or biologic false positive sera.

(2) *Fractionation.* The distribution of the reactive antibodies among the various serum components has been determined by (a) fractional precipitation with increasing concentrations of ammonium sulfate, under standardized conditions; and (b) isoelectric precipitation of the globulins by treatment of the diluted sera with CO_2. The latter method offers the advantage that it does not require dialysis and that it yields a single, strongly reactive fraction containing preponderantly *gamma* globulin (protein concentration 4–8 mg per cc). Analyses made on some 200 syphilitic and biologic false positive sera revealed that (a) fractions GI and GII, precipitable, respectively, by 1.4 and 1.7 M ammonium sulfate, contained most of the serological activity, whereas the crude albumin, remaining after precipitation of GIII by 2.1 M ammonium sulfate, was always serologically inactive; (b) the sum total of the individual titers of the isolated fractions of syphilitic sera, prepared by either of the above methods, was consistently less than that of the whole serum, whereas with biologic false positive sera, elimination of the crude albumin resulted in a significant increase in the titer of the globulins over that of the parent sera. This differential behavior, particularly pronounced with weakly reactive sera, suggested an inhibitory effect of crude albumin on the reaction of antibodies of biologic false positive sera with lipoidal antigen.

(3) *Inhibition and redispersion.* It was found that addition of the crude albumin fraction to globulin fractions derived from false positive sera causes complete inhibition of specific flocculation with lipoidal antigen, as well as redispersion of floccules formed before crude albumin was added. The addition of this fraction had little effect on the reaction of globulins isolated from syphilitic sera. So far the heat-stable inhibiting component of the albumin fraction has been found only in human sera, though individual preparations vary in potency. Crystalline human serum albumin does not exert any inhibitory effect. Further work on the isolation and identification of the inhibitor is in progress.

(4) *Heat stability.* Experiments were made with some 50 syphilitic and biologic false positive sera, or their isolated globulin fractions, by heating the samples for 20 minutes at temperatures ranging from 56 to 66° C. The data indicate that the antibodies of false positive sera are more susceptible to heat inactivation than those of syphilitic sera. The presence of non-specific serum proteins appears to be without effect on the temperature-activity relation.

(5) *Adsorption on calcium phosphate.* A considerable degree of purification of the syphilitic antibody has been achieved by adsorption from whole sera on freshly precipitated calcium phosphate, followed by precipitation of the eluate with ammonium sulfate.[7] This procedure has yielded under optimal conditions a solution containing 0.015 mg of protein nitrogen per cc per 4 flocculation units. While about 80 per cent. of the total antibody activity of syphilitic sera of varying titer is adsorbed by the calcium phosphate, in a limited number of experiments with false positive sera, no activity has been found in the eluate.

The observations described herein indicate that the antibodies of truly syphilitic sera, reactive with lipoidal antigen, differ from those of biologic false positive sera in certain chemical and immunological respects. The possibility of the application of these findings to the development of a practical method of differentiation is being explored.

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A NEW TRYPANOCIDAL AGENT: γ-(p-ARSENOSOPHENYL)-BUTYRIC ACID[1]

γ-(p-ARSENOSOPHENYL)-BUTYRIC acid was first described in a paper from this laboratory in 1940[2] It is a stable white crystalline powder, slightly soluble in water, but readily soluble in dilute alkali. Neutral solutions of the sodium salt can be sterilized by autoclaving, and such sterile solutions in sealed ampules

[7] H. Neurath, F. W. Putnam, E. Volkin and J. O. Erickson, Abstracts of papers presented at the one hundred and eighth meeting, American Chemical Society, New York, N. Y., September, 1944, p. 21B.

[1] Preliminary report.

[2] G. O. Doak, H. G. Steinman and H. Eagle, *Jour. Am. Chem. Soc.*, 62: 3012, 1940.