that when their sera are pooled after extremely careful selectivity the pool titre is 1–2,048. The selectivity includes three separate tests for activity as well as tests for specificity. In the careful preparation of these serum pools, we know that not every one of the sera used has an agglutinin titre of 1–2,048 individually. In this particular phase of the determination of the strength of the pool the specific cells used were a number of known weak group B and known A_2 subgroup.

Despite this apparently satisfactory titre it has been noted numerous times that the avidity of agglutinins does not always occur in direct relation to the number of agglutinins present in the human serum. Although the titre may show 1–2,048, the serum upon test against specific cells may result only in a moderate agglutination.

During the course of our recent investigations, we found it feasible to dry *in vacuo,* after preliminary deep freeze in dry ice, our pools of anti-A and anti-B serums. These were dried in 0.1 cc quantities, in small vials using a short drying period. We found that upon restoration with either a 1 per cent. or 5 per cent. cell suspension in 0.9 per cent. saline that the avidity of the agglutinins was more than doubled. The reaction equalled that of a serum highly concentrated by drying or a highly concentrated globulin fraction.

Inasmuch as only water is removed during the drying process, it was concluded that the alteration of the sodium chloride content was radically affecting the agglutinin strength. This was shown definitely when upon the addition of distilled water on restoration the activity of the serum was about the same as before drying, *i.e.,* moderate only. The same effect was obtained where solid HaCl was added to liquid serum in order to increase toxicity.

Tests were set up to determine the amount of sodium chloride in cell suspensions which, added to the dry serums, would impart the greatest avidity to the agglutinins. Using 5 per cent. cell suspensions prepared in 0.5 per cent. sodium chloride, 0.6 per cent., 0.7 per cent., 0.75 per cent., 0.8 per cent., 0.85 per cent. and 0.9 per cent. both anti-A and anti-B showed complete agglutination in two seconds and 4+ agglutination at 30 seconds. However, upon varying the amount of sodium chloride from 1.1 per cent. to 2.0 per cent. (by tenth per cents.) in cell suspensions, we noted a gradual lessening of avidity. At 2.0 per cent. the reaction was almost doubtful.

Therefore, at the present time we find that a final salt concentration in a range of 1.45 per cent. to 1.75 per cent. will impart the greatest avidity to the isohemagglutinins.

SUMMARY

(1) The avidity of agglutinins in human anti-A and anti-B serums is influenced by the tonicity of the menstruum.

(2) The addition of sodium chloride in final concentration in a range of 1.45 to 1.75 per cent. will greatly increase the avidity of human serum isohemagglutinins.

The author wishes to gratefully acknowledge the suggestion of Captain John Elliott (U.S.A.Sn.C.), that the restoration with hypertonic saline solution be investigated.

KATHARINE O. PATTERSON
WYETH INSTITUTE OF APPLIED BIOCHEMISTRY,
PHILADELPHIA, PA.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

CONTROL AND EVALUATION OF BLOOD SERUM ASSAYS FOR PENICILLIN

THE power of the blood of man and animals to inhibit the growth of many bacteria has long been recognized. This inhibitory power varies with species and races, but it manifests itself primarily as an individual difference either in immunity or possibly in metabolism or body chemistry. Blood of normal adults is more inhibitory than that of normal children; disease increases the titre in both. Included among the organisms inhibited by the blood of man are streptococci, staphylococci and certain aerobic spore formers, all of which are employed at present in the assay for the potency of penicillin.

For the past year[1] a serial dilution method of assay employing *Bacillus subtilis* as the test organism has been used. In investigational work on several salts of penicillin,[2] oral penicillin[3] and penicillin "X",[4] several hundred blood samples obtained at varying intervals after the administration of penicillin were assayed. A blood specimen taken from an individual prior to administration of penicillin served as a control. After a number of individuals (laboratory personnel) had volunteered as test subjects several times each, it was observed that the titre of the blood-inhibitory substances for *B. subtilis* was both variable and transitory in a given individual. It was obvious,

[1] Wm. A. Randall, C. W. Price and Henry Welch, SCIENCE, 101: 365, 1945.

[2] L. E. Putnam, H. Welch and S. Olansky, *Jour. Am. Med. Asn.*, 127: 204, 1945.

[3] H. Welch, C. W. Price and V. L. Chandler, *Jour. Am. Med. Asn.*, 128: 845, 1945.

[4] H. Welch, L. E. Putnam, Wm. A. Randall and R. P. Herwick, *Jour. Am. Med. Asn.*, 126: 1024, 1944.

therefore, that the titre of these inhibitory substances could materially affect the interpretation of the penicillin concentration of the blood sample. This would be true particularly when penicillin was present in the serum in concentrations of 0.125 unit or less. Estimation of blood penicillin levels is important in individuals undergoing treatment with the drug, and since infected persons usually give a higher titre of blood inhibitory substances than they would normally, differentiation is essential.

Addition of a penicillin inactivating substance to a duplicate dilution of the test serum, as a means of measuring the blood anti-subtilis inhibitory substances in each specimen assayed by our method, was investigated and proved satisfactory. Because of the comparatively low concentrations of penicillin present in the blood, only small quantities of the inactivating substance are required and the dilution of the serum tested is unaffected. The control series consists of a duplicate serial dilution of each serum under test by the *B. subtilis* method,[1] prepared as follows:

One-half ml amounts of broth are placed in Wassermann tubes and serial dilution by halves made by adding one-half ml of the serum being tested to one of the tubes and carrying one-half ml in serial dilution for as many tubes as necessary. The first tube in the series contains one-half ml of the serum only. To each tube is added 1.5 ml of broth containing a 1:100 dilution of a broth culture of *B. subtilis* plus the amount of sterile penicillin-inactivator necessary to destroy all the penicillin in the specimen. The standard solution (Calcium Salt reference standard) is similarly controlled and growth should occur in all tubes of this series.

We have employed two penicillin-inactivating substances in our investigation (commercial preparations of "Clarase"[5] and "Penicillinase"[6]), and have found them equally effective. The efficacy of each lot of either of these substances is established against a standard penicillin before use in the test. Since the penicillin is inactivated in the duplicate control series of each specimen, lack of growth of the organism in one or more tubes reveals the titre of the blood-inhibitory substances. If the inhibition titres of the penicillin assay series and the control series are equal, the serum under test is considered to have no penicillin activity. However, if the assay series shows one or more tubes of inhibition beyond that of the control series, it is recorded as the penicillin concentration of the serum.

In a series of 211 blood specimens collected at different intervals from 48 adults, assayed and controlled by this method, 85, or 40 per cent., have been shown to contain an anti-subtilis factor. Sixty-four of these showed inhibition, after complete inactivation of the penicillin present, in a 1:4 dilution, 15 in a 1:8, 5 in a 1:16 and one showed inhibition in a 1:32 dilution. In 16 (approximately 20 per cent.) of the 85 specimens showing anti-subtilis inhibitory substances, the titre was equal to that obtained in the test series, thus making it impossible to estimate the level of penicillin in these sera. These represented low levels of apparent penicillin concentration in the serum (less than 0.125 u/ml), and in the majority of cases were specimens collected 8 hours or more after administration of the drug. Four of the individuals were in good health (*i.e.*, not under treatment for a specific disease); the majority of the others were under treatment for gonorrhea. Of the four healthy individuals, only one showed blood inhibitory substances (titre 1:16), however, only one blood specimen was obtained from each of these persons. Of the 44 patients under treatment for specific disease, specimens from 24 (55 per cent.) contained an anti-subtilis factor.

The possibility that the anti-subtilis factors, particularly in higher titre, may have been due to the presence in the serum of types of penicillin other than "G" and "X" led us to test all available forms for inactivation by "Clarase." This was accomplished by employing 50 u/ml solutions of the following types of penicillin: crystalline "G", crystalline "X", crystalline "F" (2 lots), calcium reference standard and commercial sodium penicillin "X" (2 lots) in serial dilution, as described above, with a "Clarase" solution in the inoculum. The inactivation by "Clarase" of the seven different solutions was of the same order of magnitude. A chemical method of assay of penicillin employing the "penicillinase" indicated similarly complete inactivation of three types of penicillin ("G", "F" and "X") by that substance.

The addition to the blood specimen of a substance which neutralizes the effect of the blood-inhibitory power without interference with penicillin, thus eliminating the duplicate dilution of each serum is under investigation.

SUMMARY

A method for adequate control and evaluation of blood serum assays for penicillin is given and the effectiveness of the method for controlling the several types of penicillin is shown.

VELMA L. CHANDLER
CLIFFORD W. PRICE
WILLIAM A. RANDALL

FOOD AND DRUG ADMINISTRATION,
WASHINGTON, D. C.

[5] C. A. Lawrence, SCIENCE, 98: 413, 1943.

[6] E. B. McQuarrie and A. J. Liebmann, *Archives of Biochemistry*, 5: 307, 1944.