Penicillin Blood-Level Determinations With a *Streptococcus dysgalactiae* Resistant to Normal Blood Inhibitors[1]

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Renewed interest has recently developed in the various methods that have been proposed for the determination of penicillin levels in body fluids. Elias, *et al.* (*2*) have pointed out that the two test organisms (*Streptococcus pyogenes* C-203 and *Bacillus subtilis*) commonly employed in determining penicillin blood levels are inhibited by human sera of normal subjects and ailing cases. Their data indicate that 49 per cent of normal adult sera have inhibitory substances against the streptococcus strain and 89 per cent against *B. subtilis*. Sera from patients showed a much higher percentage of inhibition. Chandler, Price, and Randall (*1*) made the same observations and attempted to overcome the natural blood bactericides by adding penicillinase to the system. The antisubtilis factor in normal human sera was determined in our laboratory, and it was found to be present in 66.6 per cent of 41 hospitalized cases before any medication was administered. Frieden and Frazier (*3*) have reported that the inhibition of staphylococcus growth which occurs in the presence of plasma or serum is due to the globulin fraction of the blood proteins. In view of these findings a new test organism was needed for use in determining penicillin levels in body fluids.

A search was made for an organism possessing the properties of high penicillin sensitivity and resistance to the normal blood bactericides. Tests of a large number of freshly isolated strains of mastitis streptococci revealed that most of these organisms were resistant to the normal blood bactericides and that their sensitivity to penicillin varied from 0.1 unit to less than 0.05 unit of penicillin/ml. in the test broth. One of these organisms was found to be sensitive to the extent that it was inhibited by 0.0025 unit of penicillin/ml. of broth. After a series of transfers in artificial media this organism became less sensitive, finally becoming stabilized so that it was inhibited repeatedly within the range of 0.006–0.008 unit/ml. of penicillin in broth. Furthermore, it grew more luxuriantly in tryptose broth containing normal serum from human, rabbit, or bovine sources. This strain was isolated from a clinical case of bovine mastitis. By means of physiological and serological reactions it was identified as *Str. dysgalactiae*..

Technique of the test. Tryptose broth medium (tryptose, 2 per cent; dextrose, 0.2 per cent; $Na_2HPO_4 \cdot 12H_2O$, 0.2 per cent; NaCl, 0.25 per cent; pH 7.2–7.4) is used to carry out the assays in this test. The serial dilution method is used, since it is rapid, simple to execute, and its accuracy is acceptable for routine testing of penicillin blood levels (*4, 5*). Serial dilutions are carried out as follows: Tryptose broth is distributed into 10 × 120 mm. sterile tubes. The first tube receives 1.2 ml., and the remaining, 1 ml. To the first tube is added .8 ml. of the blood serum to be tested. After thorough mixing, 1 ml. of the broth serum mixture is removed and added to the second tube. The serial dilution process is continued for the desired number of tubes. A parallel series of serial dilutions is prepared in which a standard penicillin solution of 1 unit/ml. is used in place of the serum. The test organism (*Str. dysgalactiae* 245) is grown in tryptose broth for 18 to 24 hours, and 0.1 ml. of a 1:100 dilution is added to each of the tubes. The number of organisms per inoculum will vary from 100,000 to 300,000 bacteria. The tubes are then incubated at 37° C., and the results are recorded 16 to 18 hours later. The last tube showing no growth is taken as the end point. The calculations for determining the penicillin content of the unknown are made by direct comparison with the standard penicillin tubes. If the tubes containing the unknown sample reveal no growth through the seventh tube and the tubes containing the standard penicillin also show the same result, the unknown would have 1 unit of penicillin/ml. of serum. Inhibitions of the unknown above or below the seventh tube will be computed on the basis of the serial dilution principle. For example, if the eighth tube of the serum series shows inhibition, whereas the standard penicillin series shows inhibition through the seventh tube, the unknown would contain 2 units of penicillin/ml. But, if the unknown series shows inhibition through the sixth tube, the serum would contain 0.5 unit of penicillin/ml. The sample protocol in Table 1 illustrates the procedure.

TABLE 1

SERUM AND STANDARD SERIAL DILUTIONS FOR DETERMINING PENICILLIN BLOOD LEVELS

Serum Series								
Tube No.	1	2	3	4	5	6	7	8
Broth (ml.)	1.2	1	1	1	1	1	1	1
Serum (ml.)	0.8	.....	dilute serially	.....				
Culture, 1:100 (ml.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Standard Penicillin Series								
Tube No.	1	2	3	4	5	6	7	8
Broth (ml.)	1.2	1	1	1	1	1	1	1
Penicillin solution, 1 unit/ml. (ml.)	0.8	.....	dilute serially	.....				
Culture, 1:100 (ml.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

[1] The authors wish to express their appreciation to Mary A. Medill for her technical assistance.

Results. Using the technique described above, 30 human sera obtained from adult patients and normal subjects were tested for the presence of inhibitory substances against *Str. dysgalactiae*. Six bovine and two rabbit sera were tested also. No inhibition was demonstrated by any of the sera. In fact, the organism grew more luxuriantly in the tubes containing 1:2.5 serum dilution than in the tubes containing less or no serum.

TABLE 2

PENICILLIN BLOOD LEVELS OF PATIENTS RECEIVING PENICILLIN INTRAMUSCULARLY

(Test organism: *Streptococcus dysgalactiae*)

Patient	Dosage	Administration intervals	Blood levels		
			1 hr.	2 hr.	3 hr.
	Penicillin units	*Hr.*	*Penicillin units/ml. serum*		
1	20,000	3	0.25	0.06	0.016
2	30,000	3	0.13	0.03	0.016
3	40,000	3	0.13	0.016	
4	20,000	4	0.03	0	0
5	20,000	3	0.06	0.016	0
6	30,000	3	0.13	0.06	0.016
6	30,000	2	4.0*	2.0	
7	30,000	3	0.06	0	0
7	30,000	2	1.0	0.25	
8	30,000	2	0.25	0.25	

* This patient developed uremia with urinary retention.

This procedure was also applied in eight human hospitalized cases undergoing penicillin therapy. The results of the penicillin blood levels are shown in Table 2. Although the number of cases reported in this series is small, it will be seen from these results that, in the cases reported here, the penicillin blood levels are not therapeutically adequate when 20,000 units are given intramuscularly at three-hour intervals.

Summary. A strain of *Str. dysgalactiae* was found to be an effective test organism for penicillin blood-level determinations. This organism, although inhibited by penicillin in concentrations of 0.006–0.008 unit/ml., is resistant to the natural inhibiting substances of blood sera. The latter characteristic is very significant, since the test organisms (*Str. pyogenes* C-203 and *B. subtilis*) that are now employed for penicillin assay of body fluids are inhibited by a large percentage of human sera.

The method described can detect penicillin blood levels in concentration of 0.016 unit/ml. of blood sera. Since blood levels above 0.03 unit/ml. are considered to be therapeutically effective, this method of assay is adequately sensitive for routine clinical application.

References

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Letters to the Editor

Taxonomy and the Biologists

Carleton R. Ball's recent communication (*Science*, 1946, **103**, 713) states the grievances of the nontaxonomists so cleverly that they are apt to be accepted at full face value. These statements, however, are only partially valid. Systematists of today are not primarily interested in describing new species, or in erecting new names to replace old ones merely for the purpose of having their names attached to these supposedly new forms. Their primary motive is a sincere desire to place before the other workers in biology as full and complete a record of the forms living in the world as is possible with our present support and opportunities.

That he is doing as good a job in his field as the workers in any other field is a challenge that must stand until someone produces reliable statistics to the contrary. The mere listing of the mistakes made by the taxonomists will not override the challenge, because mistakes are made in all fields. The chief difference is that the taxonomist is the only worker who embalms his mistakes and erects them like totem poles along the highway, so that each succeeding generation of taxonomists must do obeisance as they pass by. Unfortunately no one has proposed a real remedy for this burdensome process.

Changes in generic names are due chiefly to five things: First is the fact that some earlier systematist has described the genus under another name. With the present survey of literature nearly complete, this cause for name-changing is almost a thing of the past. Second is the discovery that two authors have used the same name for two different organisms. With the recent publication of Neave's *Nomenclator Zoologicus*, most of the previous duplications can be cleared up. The number of duplications in the future should be small with such world-wide reviews of current literature as are now being published. The third cause of confusion is due to present and past methods of type selection. However, with strict enforcement of a rule which would prevent publication of new generic names without clear type designation, such confusion should be reduced to a minimum. Fourth is the division of a genus into two or more genera. This process has continued since the beginning