

Department of Internal Medicine, University of Berne, Switzerland
(Dir. Prof. W. Hadorn)

SEROLOGIC STUDIES ON SERUM OF DRUG ALLERGIES WITH A POLYSTYRENE-LATEX- TECHNIC MEASURED BY NEPHELOMETRY

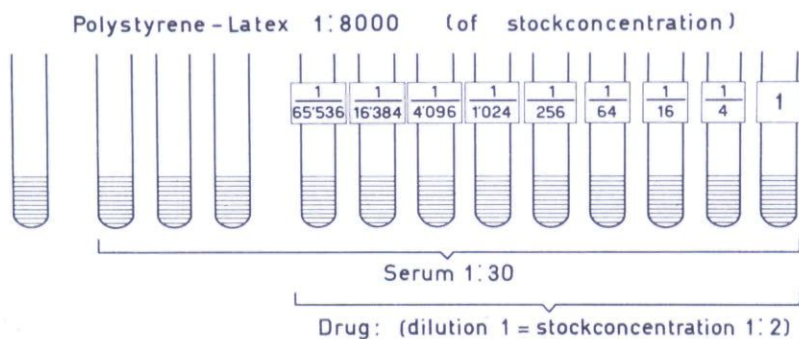
By

R. HOIGNÉ, M. JAEGER & R. GAUTIER-HUNTER

Different methods have been used in a number of research laboratories to show specific reactions of serum of allergic patients with a known allergen or hapten. Some of these technics gave interesting results in the hands of a limited number of investigators. The ideal of a simple and easy reproducible technic has, however, not yet been attained. The technics employed in our laboratory seem to show a reactive principle which is present in serum only in a small group of clinical drug reactions for a relatively short period of time (12, 13).

A new approach to in vitro studies on sera of drug-allergies consists in combining a particle technic and nephelometric measurement. Collodium pellets were used by earlier investigators to increase serologic reactions (Loeb, Jones, Freund, Delves, Cannon and Marshall, Goodner, Eisler, Cavelti, Jiménez-Díaz et al., Hässig and Lüscher, Kallós and Kallós-Deffner). In these experiments however, polystyrene-latex has been applied, the same particles that Singer and Plotz proposed for the serum diagnosis of rheumatoid arthritis (21). In our experiments instead of visible partical agglutination, turbidity changes were determined. The photoelectric

This work was aided by a grant from the "Swiss National Foundation for Scientific Research" and from the "Sandoz-Stiftung zur Förderung der medizinisch-biologischen Wissenschaft".



Stock concentrations:

Polystyrene-Latex: 11% of dry material

Drug: One daily dose diluted in 1 liter of buffered isotonic sodium chloride

Fig. 1.

measurements, were based on own experience with nephelometry on sera of drug allergic patients. (11). The technic as originally described had to be modified in some ways to exclude false positive reactions (3, 12), but its disadvantage could not be eliminated, namely that of extremely small turbidity changes when applied to non protein "antigens" (13). Polystyrene-latex seems to be of some help in increasing the reaction.

The new technic will be explained with the help of some slides. The method has been described in detail elsewhere (14).

The preparation of polystyrene-latex and its addition of the drug and serum is shown in *figure 1*.

From a stock solution of polystyrene-latex containing 11 % of dry material, a dilution of 1:4000 in buffered,¹ isotonic sodium chloride is prepared. Large particle agglutinations are removed by slight centrifugation (1500 r.p.m. for 5 minutes).

The stock solution of the drug contains one daily dose in one

¹ Na Cl 0.9 % 3 parts with phosphate puffer of Sørensen 1 part.

1. Polystyrene - Latex + Drug in buffered isotonic Na Cl
12-24 hours

2. Serum Additions: 1 hour after withdrawal of blood

3. Nephelometric Measures:

I Before serum addition

II 30 minutes after serum addition

III 1½ - 2 hours after serum addition

Fig. 2.

liter of physiologic, buffered saline, of which the dilutions 1:4, 1:16 etc. up to 1:65 536 are prepared.

By mixing equal amounts of polystyrene-latex standard dilution and drug solutions, a final polystyrene-latex concentration of 1:8000 is obtained.

The first tube of the series is used as the standard for the nephelometer; it contains only polystyrene-latex with physiologic saline. Tubes 2-4 are prepared in exactly the same way. They later receive 0.1 ml of serum as do the tubes containing the drug dilutions. The addition of 0.1 ml of serum to 3.0 ml of polystyrene-latex with allergen, yields a constant serum concentration of 1:30 in each tube.

The time intervals used in the methodical studies are shown in *figure 2*.

Polystyrene-latex and allergen or the control solution is kept over night at room temperature. After these 12-24 hours, which should permit adsorption to take place, the original turbidity of each tube is measured in one and the same nephelometric cuvette (14). The photocolormeter Elko II of C. Zeiss with nephelometer-attachment has been used. (To allow accurate measurements, only a series with turbidity changes of less than $\pm 1/3$ of the absolute turbidity of the first tube should be used (21)).

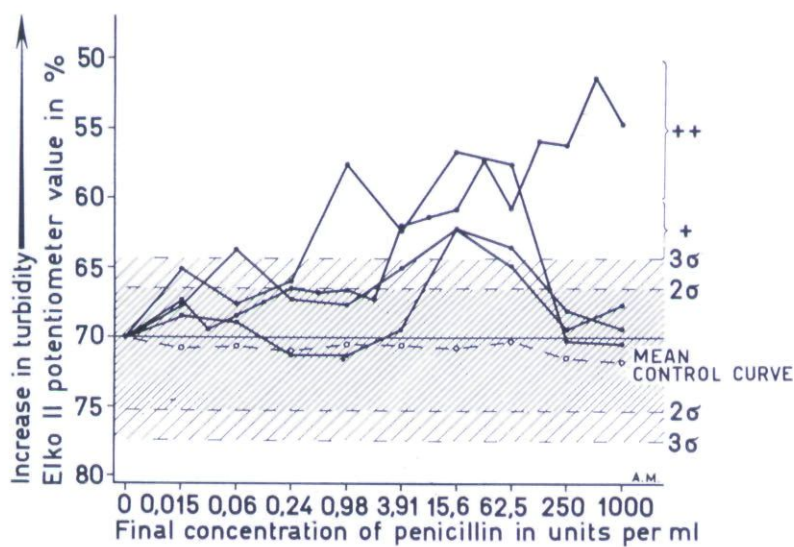


Fig. 3.

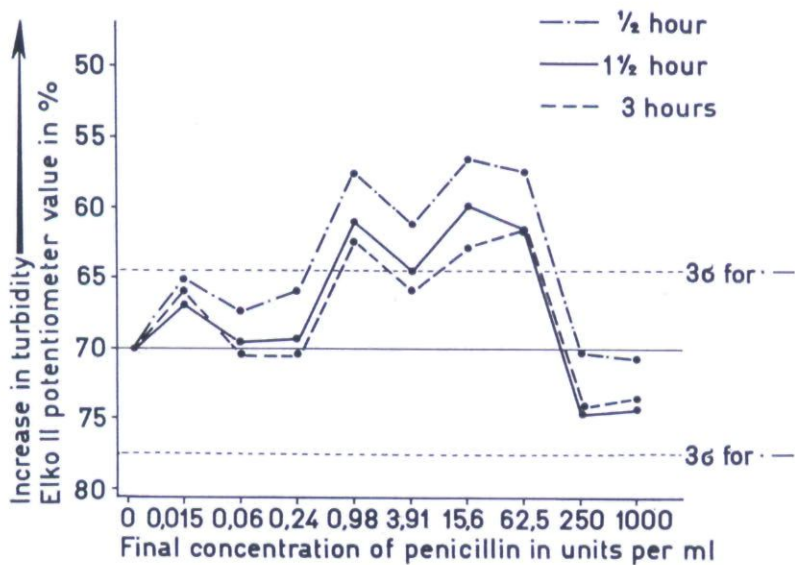


Fig. 4.

After the addition of serum the contents of each tube, even the first without serum, is stirred 10 times with a glass rod. The second nephelometric measurement of the tubes is performed 30 minutes later. Further measurements may be made after 1, 2, 4 and more hours.

The change in turbidity of each tube is the difference between the readings before and after serum addition. It is, for simplicity's sake expressed in units of the nephelometer. With the help of a turbidity standard these can be converted into measurements of absolute turbidity (21). Later on the turbidity effect of the serum on polystyrene without drug (tube 2-4) is subtracted.

Figure 3 shows the turbidity diagrams of sera of four patients with a clinical reaction to penicillin-procaine. The curves with penicillin considered to be positive have the following characteristics:

1. The optimal increase in turbidity is greater than 3-times the standard deviation of 17 control sera.
2. There is also an increase of turbidity in drug concentrations bordering the one of maximum reaction.

Repeated measurements on the same tubes show the reproducibility of the general shape of the curve (*Figure 4*). Turbidity readings obtained one or more hours after serum addition may no longer yield the same significant turbidity change (*Figure 5*).

Sera of patients were tested at different time intervals after the clinical reaction to the drug. A positive reaction could only be found within 6 days of drug withdrawal. In the patients with an anaphylactoid shock or exanthema due to penicillin-procaine, only penicillin was positive, never procaine. In a single case, the reaction could be demonstrated on blood drawn on three different days. Reexposure of the patient was considered to be dangerous with penicillin and procaine, but the observed clinical reaction followed in 3 of these 4 instances minutes after the penicillin-procaine injection.

Similar invitro reactions as with penicillin were observed

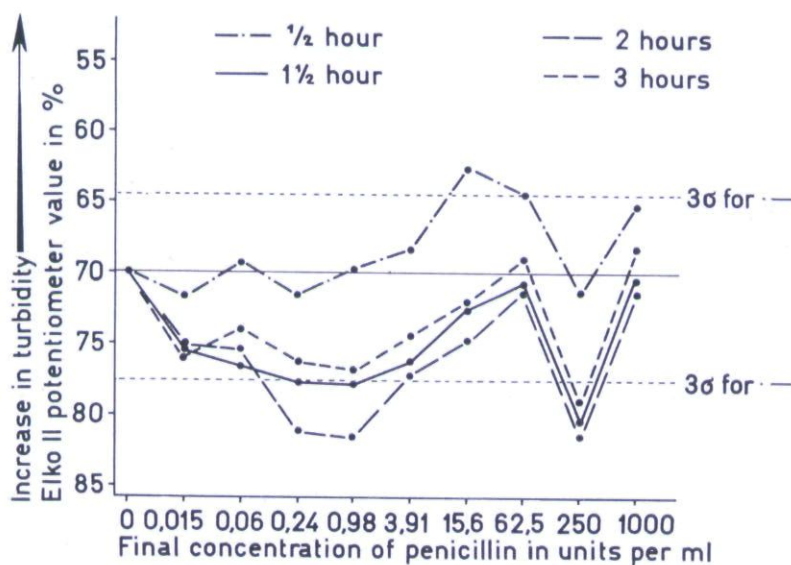


Fig. 5.

in four cases with proven sensitivities to simple chemical compounds such as quinine, mesantoin, a sulfonamide and a thio-uracil preparation. Re-exposure of these patients to the drug confirmed the serologic result.

In many other patients with drug allergy no positive reactions were found even when the test was repeated several times.

Further studies of the reaction should be carried out to prove specificity and to find ways of increasing even more the turbidity change. It may be, that more pronounced reactions would permit identification of the responsible drug in a higher proportion of patients.

The positive reaction was never strong enough to be read by the naked eye, neither immediately nor after spending 24 hours at room-temperature.

The centrifugation-resuspension technic with macroscopic reading of the agglutination was used only in a restricted number of cases and gave negative results (1, 8).

The nephelometric measurement is felt to be a sensitive and objective method, if each result is evaluated statistically, comparing the reaction with numerous control sera for the same allergen (6). To prevent false positive reactions, a constant sensitivity of the nephelometer and small variations of the original turbidity of the tubes in the same series are of fundamental importance. Sera of patients with rheumatoid arthritis or liver disease were excluded from this study.

It is of great interest, that Ley describes also in this year, a highly specific factor in some sera of patients sensitive to penicillin, which is able to agglutinate penicillin treated erythrocytes. The reaction observed by Ley seems to be extremely specific. The thrombocyte test in vitro, previously described indicated similar results (10), but it was put aside as the technic is extremely delicate.

Loiseleur found an increase in serum viscosity, if penicillin was added to the serum of patients during or shortly after penicillin therapy. This phenomenon occurred also in patients who did not show clinical reactions to this drug (20).

REFERENCES

1. Cannon, P. R. & Marshall, Ch. E.: J. Immunol. 38, 365, 1940.
2. Cavelti, P. A.: J. Immunol. 49, 365, 1944. 57, 141, 1947.
3. Cormane, R. & Zwart Foorspuij, A. J.: Schweiz. med. Wschr. 86, 413, 1956.
4. Delves, Edna: J. infect. Dis. 60, 55, 1937.
5. Eisler, D. M.: J. Immunol. 42, 405, 1941.
6. Fisher, R. A.: Statistical methods for research workers (Oliver and Boyd, Edinburgh and London 1948).
7. Freund, J.: Proc. Soc. exp. Biol. (N.Y.). 28, 65, 1930.
8. Goodner, K.: Science 94, 241, 1941.
9. Hässig, A. & Lüscher, E.: Schweiz. Z. Allg. Path. u. Bakt. 17, 440, 1954.
10. Hoigné, R. & Storck, H.: Schweiz. Z. Allg. Path. u. Bakt. 17, 440, 1954.
11. Hoigné, R., Grossman, W. & Storck, H.: Schweiz. med. Wschr. 85, 578, 1955.
12. Hoigné, R.: Symposium on Sensitivity Reactions to Drugs. WHO, Liège 1957.
13. Hoigné, R., Huber-Stoller, E., Coley, E., Rodriguez, F. & Isliker, H.: Schweiz. med. Wschr. 88, 331, 1958.

14. *Hoigné, R.*: *Helv. med. Acta.* 25, 422, 1958.
15. *Jimenez-Diaz, Arjona, E., Alés, J. M. & Segovia, J. M.*: *Bull. Inst. med. Res. (Madr.)*. 3, 125, 1950.
16. *Jones, F. S.*: *J. exp. Med.* 46, 303, 1927. 48, 183, 1928.
17. *Kallós, P. & Kallós-Deffner, L.*: *Int. Arch. Allergy*, 2, 70, 1951. *Antibiot. et Chemother. (Basel)*. 3, 145, 1956.
18. *Ley, A. B., Harris, J. P., Brinkley, M., Liles, B., Jack, J. A. & Cahen, A.*: *Lancet*. 127, 1118, 1958.
19. *Loeb, J.*: *J. gen. Physiol.* 5, 109, 479, 1922.
20. *Loiseleur, J.*: *Biologie Médicale*, 47, 3, 259, 1958.
21. *Sauer, H.*: *Z. techn. Physik*. 12, 148, 1931.
22. *Singer, J. M. & Plotz, Ch. M.*: *Amer. J. Med.* 21, 888, 1956.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.