NOTES

LATEX AGGLUTINATION-INHIBITION TEST FOR SCREENING
BLOOD-CLOTTING FACTOR XIII IN HUMAN PLASMA*

For the assay of blood-clotting Factor XIII (Fibrin-Stabilizing Factor, FSF), the antibody-neutralization test (Bohn and Haupt, 1968), the monodancylcada-verine incorporation test (Lorand et al., 1969; Nishimura et al., 1975) and the Laurell method are tried in our country (Hidano et al., 1975). None of them, however, could be applied to the clinical screening for Factor XIII-deficient cases; the development of a simple and rapid test procedure has been awaited for.

We found that Factor XIII contained by the concentrate prepared by the Behring Institut was adsorbed to latex particles, which, then, were specifically agglutinated by rabbit anti-human Factor XIII antibody also supplied by the Behring Institut. When normal human plasma was added to the antibody prior to mixing the antibody with the antigen-coated latex particles, agglutination was no longer observed, indicating that the antibody had been consumed by its binding to the Factor XIII present in normal human plasma. For excluding any reaction attributable to subunit S, which is said to be still present in human serum after clotting had taken place and also in Factor XIII-deficient plasma (Bohn et al., 1972), experiments were performed exclusively by using specific antiserum against subunit A which bears Factor XIII activity.

A stock suspension of latex particles (No. 15, 0.85 μ, 4.1%) supplied by Takeda Pharmaceutical Co. (Osaka, Japan) was diluted 1:10 in 0.01 M PBS (pH 7.3); it was mixed with an equal volume of Factor XIII concentrate diluted 1:5 with the same buffer. The mixture was incubated at 37 °C for 2 hr. Then the latex particles were washed three times with PBS and resuspended to about 1%.

A 0.05-ml portion of an IgG-containing fraction from rabbit anti-subunit A serum was mixed with the equal volume of a test sample and the mixture was kept at room temperature for 10 min. Then, 0.1 ml of the mixture was mixed with the equal volume of the latex reagent (antigen-coated latex particles) on a black plate. The plate was gently rocked and examined macroscopically for the presence of visible agglutination. When visible agglutination was observed within 5 min, it was interpreted as being uninhibited by the test sam-

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It was found convenient to store the antibody as an IgG-containing fraction obtained by rivanol treatment instead of whole antiserum to avoid deposit formation interfering with the test results. The method of rivanol treatment modified from the one described by Horejsi and Smetana (1956) with human serum was applied to the rabbit antiserum. 1.68% rivanol was added to an equal volume of rabbit serum and the mixture was kept at room temperature for 2 hr. The supernatant fraction was obtained by centrifugation (3,000 rpm for 10 min). Rivanol was precipitated from the supernatant fraction by addition of sodium chloride to a final concentration of 5%. The fraction thus obtained was dialyzed overnight. The final protein content was 0.5 g/dl. Recovery of IgG into the supernatant fraction was usually higher than 80% (Masuda and Yasuda, 1975). It also contained albumin, transferrin, IgA, hemopexin, β2-glycoprotein and a trace of IgM but no α2-macroglobulin.

It is said that various human plasma proteins were added to the Factor XIII concentrate available from the manufacturer. But it was not difficult to set up the condition under which the agglutination was specifically inhibited by undiluted human plasma, but not serum. This purpose was attained by controlling the concentration of the antibody and the degree of coating of latex with the antigen. The dilution of Factor XIII concentrate just allowing inhibition of agglutination with undiluted plasma was found to be 1:80. Namely, the Factor XIII concentrate was 80 times higher in its antigenic titer than normal human plasma. The antibody fraction prepared as described above was usually diluted 1:4.

Next, when our results with 15 individual human plasma samples were compared with the results of the monodancylcadaverine (MDC) incorporation test performed at the First Department of Medicine, Teikyo University Medical School, none of the samples with Factor XIII activity less than 100% completely inhibited agglutination of antigen-coated latex by corresponding antibody (Fig. 1). Thus, there was agreement between the results obtained in both of the tests performed. Our latex agglutination-inhibition test requires much less time and no complicated procedures as does the MDC incorporation test, and therefore seems more easily applicable to the purpose of screening for Factor XIII-deficient cases.

With another sample from a congenital Factor XIII-deficient case kindly supplied by Dr. K. Nakamura, First Department of Medicine, Tottori University Medical School, inhibition was not observed, either. From our results thus far obtained, this agglutination-inhibition test can be used in screening for Factor XIII-deficiency cases.

When a mixture of 0.05 ml of human thrombin (40U/ml) and 1.6 ml of 1:16 diluted Factor XIII concentrate was incubated at 37 C and its aliquots were
tested every 15 min by the latex agglutination-inhibition test, no decrease in Factor XIII antigenicity was observed for 1 hr. Moreover, with the same volume of thrombin diluted with 0.1 M Veronal- HCl buffer (pH 7.3) containing CaCl₂, the antigenicity did not decrease for 1 hr. Therefore, it can be seen that even when Factor XIII is activated, no such decrease occurred in its antigenicity that could be detected by the latex agglutination-inhibition test. These findings are in contrast, on one hand, to the paradoxical observation by Miura (1975) in the immunodiffusion technique that even human serum formed a clear precipitation band against anti-subunit A serum; on the other hand, to the findings by the staff of the First Department of Medicine, Teikyo University (personal communication) that Factor XIII antigenicity in the Laurell method disappeared upon its activation by thrombin and calcium ion.
In our experiments, only latex No. 15 supplied by Takeda Pharmaceutical Co. has been used. This was found very stable and free from nonspecific aggregation when used by Ishiyama et al. (1975) for coating with human anti-HBs antibody. Further study is undertaken to see if other latex particles can also be used for this purpose.

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REFERENCES


