FACTOR V ACTIVITY IN BLOOD DONORS

By S. B. Kwa, M.B., M.R.C.P. (Edin) M.R.C.P. (Glasg.)

and

Y. F. Chen

(Singapore Blood Transfusion Service, General Hospital, Singapore, 3)

The discovery of a case of congenital Factor V deficiency (see report in this Journal) made it necessary to develop a method for the quantitative estimation of Factor V activity in blood. This was necessary as studies of Factor V activity in blood do not form part of the routine in the average haematological laboratory. Further, as it was intended to study the levels of Factor V in the patient following transfusions of fresh frozen plasma a simple yet sensitive method of assay which could give reliable and readily reproducible results was essential.

It is now believed that the "labile factor" of Quick (1943), the "Ac-globulin" of Ware and Seegers (1948) and "proaccelerin" of Owren (1950) are one and the same factor, Alexander and Goldstein (1952). Further, that this factor by international agreement is now known as Factor V. Factor V has a definite role in the coagulation mechanism of blood, being required for both the intrinsic and extrinsic systems for thromboplastin formation. More specifically, it is necessary for the rapid formation of thrombin from prothrombin in the presence of thromboplastin derived from blood, tissue or Russell's Viper Venom, Biggs and Macfarlane (1962).

Its definite role in hemostasis is obscure though a deficiency of this factor can result in a derangement of the hemostatic mechanism producing a clinical picture not unlike mild hemophilia.

A number of methods for the assay of Factor V have been described, Quick and Stefanini (1948), Stefanini (1950), Wolf (1953), Mustard (1957), Borchgrevink and Stormorken (1960) and Quick (1960), some of which require the use of reagents difficult to prepare and standardise, or of purified clotting factors which are not readily available.

This paper describes a method for the assay of Factor V making use of reagents which are easily prepared and standardised. The Factor V levels of 41 normal male blood donors were determined. For comparison, the levels of Factor V in a further 50 normal male donors were determined by the method described by Mustard (1957).

PRINCIPLE OF THE TEST

Almost all assays so far described make use of the role of Factor V in the extrinsic thromboplastin formation system. In this system tissue factor as supplied by brain extract reacts with Factors V, VII and X to produce extrinsic thromboplastin. This then reacts with prothrombin in the presence of calcium to produce thrombin which in turn reacts with soluble fibrinogen to produce insoluble fibrin. This system is tested by the Quick's one stage prothrombin time, originally introduced as a test of prothrombin activity, but now accepted to measure in addition all the factors mentioned above.

If in such a system optimal amounts of tissue extract, prothrombin, Factors VII and X, calcium and fibrinogen are present, then the clotting times will depend on the concentration of Factor V. A reagent containing all the factors but deficient in Factor V can be artificially prepared by storing oxalated plasma at 37°C for about 48 hours. The lengthened prothrombin times of such a plasma is shortened by the addition of Factor V and the degree of shortening is proportional to the amount of Factor V added.

MATERIALS

FACTOR V DEFICIENT PLASMA (AGED PLASMA)

This is prepared by taking venous blood into one tenth volume of M/10 sod. oxalate. The plasma is then separated and stored at 37°C for about 48 hours. The one stage prothrombin times of the aged plasma should be in the region

of 50 to 70 seconds. The aged plasma is then divided into 5 ml. portions, sealed in glass ampoules and stored at minus 30°C for future use.

STANDARD NORMAL PLASMA

Although there is a wide variation in the Factor V levels in the plasma of different individuals, it is probable that like anti-haemophilic globulin levels, the Factor V level for an individual is genetically determined and relatively constant, Quick and Stefanini (1948). For this reason, plasma from the same individual is used throughout the studies as the standard reference plasma for all assays.

To avoid any possible diurnal variations, the blood was collected at approximately the same time each morning just prior to the preparation of the standard reference curve.

Venous blood from the individual is taken into one tenth volume of 3.8% sod. citrate. The blood is then centrifuged without delay at 1500 r.p.m. for 5 minutes. The supernatent plasma is removed into a clean glass tube. If the test is not to be carried out immediately, the plasma is stored in a refrigerator at 4°C.

Thromboplastin: An extract of rabbit brain (Difco) is used as a source of thromboplastin for all the assays.

Calcium: M/40 Calcium chloide is used.

Test Plasma: The plasma for assay of Factor V activity was collected and prepared in exactly the same way as the standard normal plasma.

PREPARATION OF STANDARD REFERENCE CURVE

The one stage prothrombin times of the Factor V deficient plasma and standard normal plasma were determined in duplicate and the moan of the readings taken as the clotting times representing 0% and 100% Factor V activity..

Dilutions of 5, 10, 20 and 40% of normal plasma in aged plasma are made. The prothrombin times of the dilutions are determined as above. A standard reference curve (Figure 1) is then drawn showing the clotting times in seconds against the Factor V concentration in the dilutions of normal plasma. New standard reference curves are prepared each day.

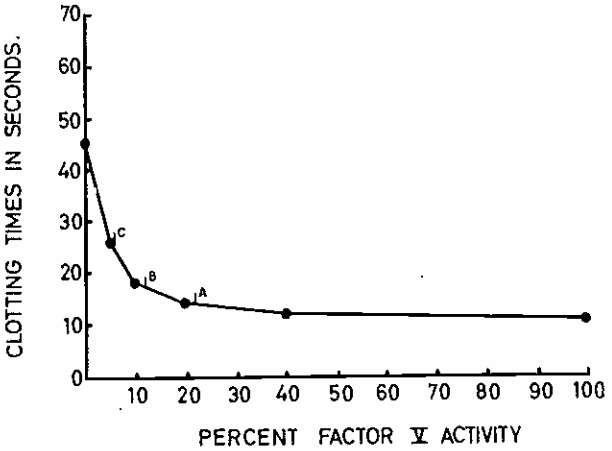

Fig. 1. A typical standard reference curve. A, B, C represent the clotting times of 20, 10 and 5% dilutions of the test plasma. These correspond to 22%, 12% and 6% of Factor V activity respectively on the reference curve. Therefore the Factor V activity in the undiluted plasma represented by A, B, and C would be 110%, 120%, 120% respectively, giving a mean value of 117% of Factor V activity.

ASSAY OF FACTOR V ACTIVITY IN TEST PLASMA

The prothrombin time of the test plasma was first determined. If it is in the normal range, then dilutions of 5, 10 and 20% of the test plasma in aged plasma are made and the prothrombin times of the dilution determined. By reference to the standard curve, the percentage of Factor V activity corresponding to the clotting times can then be ascertained and the level of Factor V in the undiluted test plasma can then be calculated. The mean of the Factor V levels obtained from the three different dilutions was then recorded as the Factor V level of the test plasma.

The choice of which dilution to use depends on the level of Factor V in the test plasma. The dilutions are so made as to give clotting times in the most sensitive range of the curve, *i.e.* between 5% and 40% of Factor V activity.

RELIABILITY OF STANDARD REFERENCE CURVE

Using the same individual as the source of normal plasma and the same batch of thromboplastin and aged plasma, standard reference curves are prepared daily.

Fig. 2 shows the results of three such curves chosen from the extremes of values obtained

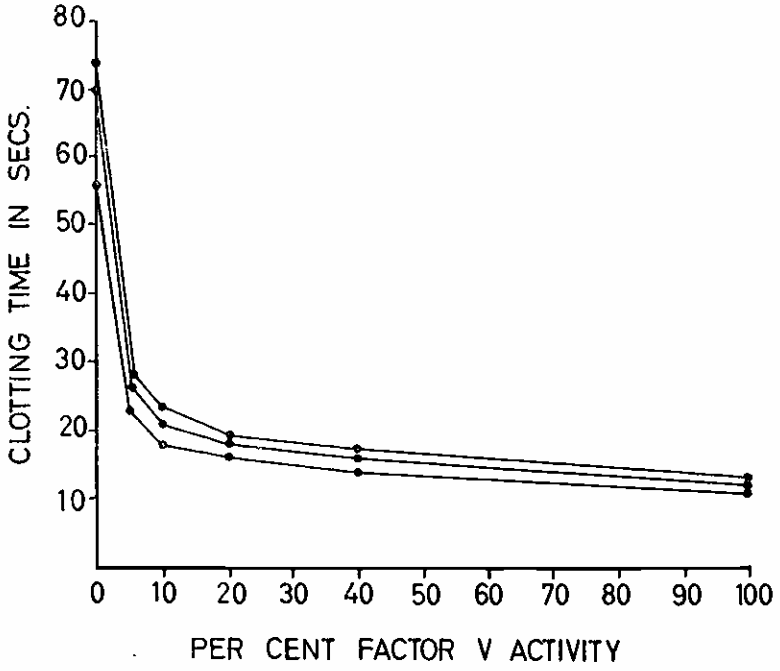

Fig. 2 Three standard reference curves prepared, on different occasions using fresh plasma from the same individual, all other reagents being the same.

during the study. The general pattern of the curves obtained on different occasions is similar. With levels of Factor V activity above 40% the curve is flat and below 5% the curve is almost vertical. The most sensitive range of Factor V activity is between 5% to 40%. Below 5% a fall of Factor V activity of 1% can give a wide range of clotting times. Above 40% a fall of 1 second in the prothrombin time gives a Factor V level of anything between 50-100%. From the results it would appear that all the other reagents being standardised and constant, the daily Factor V level of the standard normal plasma is relatively constant.

ASSAY OF FACTOR V ACTIVITY IN BLOOD DONORS

Fig. 3 shows the frequency distribution of the levels of Factor V activity in 41 normal male blood donors all of whom have passed the preliminary screening tests and medical examination before being accepted as donors. The blood from all these donors was collected in the morning after a light breakfast. The blood was collected and prepared as described and the Factor V levels assayed according to the method described.

As comparison, the Factor V levels of a further 50 normal male blood donors were assayed according to the method described by Mustard (1957). Fig. 4 shows the results using this method.

RESULTS

Using the method described, it was found that the levels of Factor V in the blood of the 41 blood donors ranged from 75% to 216% of normal, with a mean value of 132%. The majority however had levels between 80% to 180%. There is a wider range, 65% to 350% with a higher mean value of 153% when using the method of Mustard (1957). A good number of donors had values of over 220%. One of these had on repeated examination a Factor V level of 350% of normal.

DISCUSSION

So far there have been few reports of the normal range of Factor V activity in normal subjects. Quick and Stefanini (1948) making a comparative study of the concentration of labile factor in the human, dog and rabbit blood noted the relatively low concentration of labile

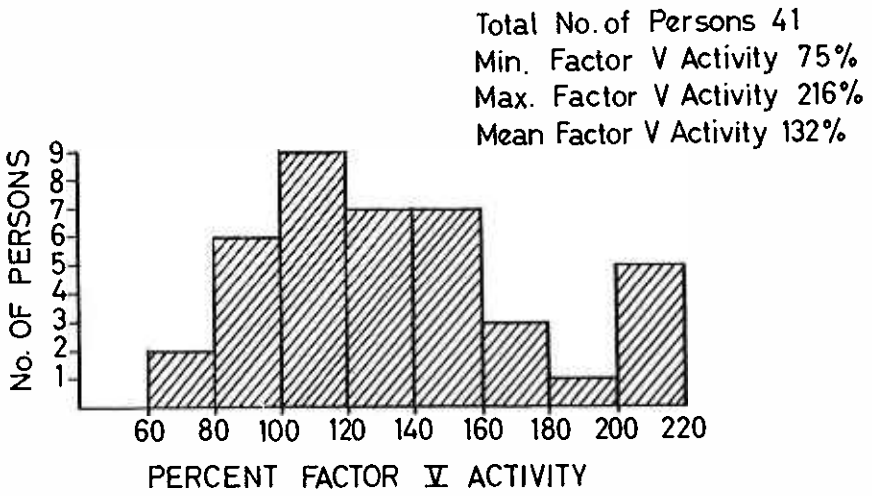

Fig. 3. Showing range of Factor V activity in 41 normal blood donors.

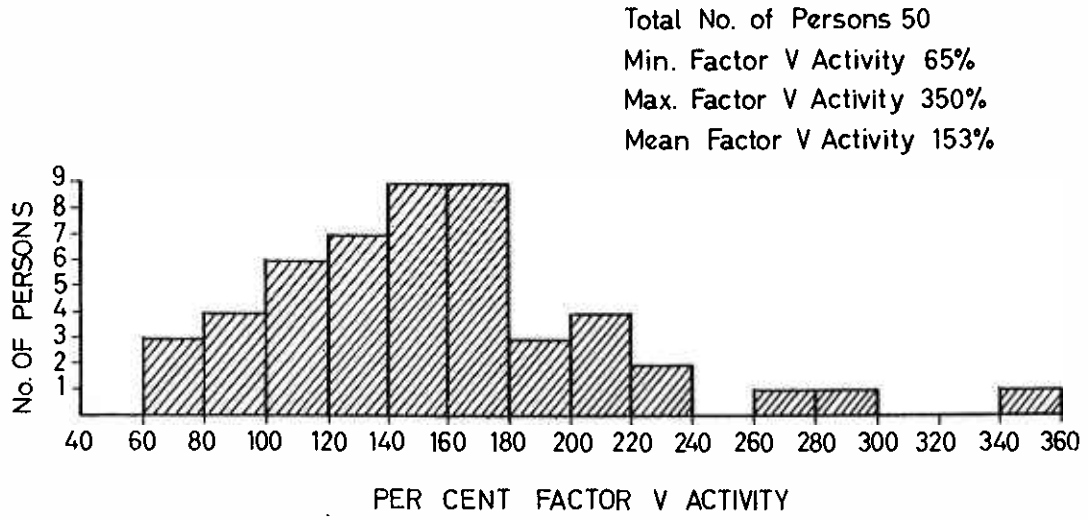

Fig. 4. Showing range of Factor V activity in 50 normal blood donors estimated according to the method described by Mustard (1957).

factor in human blood; rabbit blood containing 50 times and dog blood 10 times as much labile factor as human blood.

The inavailability of a standardised preparation of pure Factor V has meant that all assays of Factor V activity must be a comparison of the unknown to an arbitrary standard, usually obtained by pooling blood from a number of normal healthy subjects. All results would therefore only be relative.

In a similar study on 46 blood donors, Borchgrevink, Poole and Stormorken (1960) found an almost even distribution around 100% with a narrow range of 60-160%.

From the studies carried out, the values obtained on 41 donors ranged between 75% to 216% with a mean of 132%. This wide range for normal is not entirely unexpected in a biological assay of this nature. For example, in carrying out anti haemophilic globulin assays, the normal range is reported as 50% to 220% by Pitney (1956). The value of the assay lies in the observation that no normal subjects were found with a level of Factor V activity below 60%.

The high mean values obtained in this study would suggest that the plasma used for the standard reference plasma had a lower than average Factor V activity.

The wider range of 65% to 350% of Factor V activity obtained in 50 other blood donors assayed by the method of Mustard (1957)

is more difficult to explain. Mustard adopted as 100% Factor V activity the prothrombin clotting time of a 20% dilution of fresh aluminium hydroxide treated oxalated plasma in aged plasma. It is possible that by using plasma that has been diluted the test has been made more sensitive to minor changes in concentration of Factor V activity, especially at the higher levels. This could explain the greater number of donors found to have Factor V levels above 200% thereby giving the higher mean value.

Using this method we have been able to make a study of changes in Factor V level following transfusions of fresh plasma in a patient with Factor V deficiency.

SUMMARY

A method for the assay of Factor V activity in plasma has been described. The levels in 41 donors normal male blood donors was found to range from 75% to 216% with a mean level of 132% of normal. Using the method of Mustard (1957) on a further 50 donors gave a wider range and a higher mean value.

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