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TURNOVER OF PROTHROMBIN, FACTOR VII AND FACTOR IX IN A PATIENT WITH HEMOPHILIA A

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Clotting factors have a more rapid turnover than most other plasma proteins. This is often explained by assuming that coagulation is a continuous process in the normal body. However, a previous report from this laboratory (Hasselback & Hjort, 1960), showed that blocking of such hypothetical coagulation by a continuous infusion of heparin did not change the turnover rate of prothrombin and proconvertin (factor VII). The turnover was determined from the rate of disappearance of these factors from plasma after a large dose of Warfarin, which blocks their synthesis in the liver (Frick, 1958).

In this study we have used the same technique to determine the turnover of prothrombin, proconvertin and antihemophilia B factor (factor IX) in a patient with severe hemophilia A. For comparison, the experiment was repeated with identical technique in two healthy persons.

Case report.

The patient, S.B., was a 17-year-old male. His maternal grandfather and the grandfather's three brothers were bleeders. The son of the mother's first cousin is also a severe bleeder.

The bleeding tendency had been severe since infancy. Joint bleedings (ankles, knees, hips, elbows) had been frequent and disabling; x-ray examination of the knees showed marked hemophilic arthrosis. In December 1954 a fracture of the left femur led to his tenth admission to the University Clinic. The fracture healed very slowly, and he stayed in the hospital for nearly a year. During this year, he had several periods with apparently spontaneous joint and muscle bleedings.

The present experiment was performed in a good period. There was no anemia, and the clinical examination revealed nothing abnormal, except for the hemophilic joints. Table I gives a summary of the "hemostatic tests". Similar results have been obtained repeatedly over the last several years.

MATERIALS

Proconvertin reagent was prepared by the method of Hjort, (1957). It is an eluate of human serum containing proconvertin and Stuart factor (factor X), but less than 1 per cent of prothrombin. The batch used contained about 30 per cent of Stuart factor, as assayed in a one-stage system with Stuart factor deficient plasma as substrate.

Warfarin sodium. Coumadin sodium (Endo, Richmond Hill, N.Y.) for intravenous use was dissolved in distilled water to a concentration of 25 mg per ml.

The materials used for the clotting factor assays are described in detail in the references given in the next section.

Table I. *"Hemostatic tests" in patient B.S.*

Test	Patient B. S.	Normal	References
Whole blood clotting time	25–28½ min.	2.7– 7.0 min.	Hjort & Stormorken (1957)
Bleeding time	4½–5½ »	2 –12½ »	Hjort & Stormorken (1957)
Platelets per mm³	264 000	150 000–337 000	Nygaard (1933)
Cephalin time	256 sec.	57–64 sec.	Egeberg (1961)
Quick time	17.5 »	13–15 »	Quick system with human brain thromboplastin
Proconvertin	58 %	80–120 %	Aas (1952)
Prothrombin	82 %	80–120 %	See text.
Proaccelerin	107 %	80–120 %	Owren (1947)
Antihemophilia A factor	<1 %	60–150 %	Egeberg (1961)
—»— B »	73 %	70–140 %	Egeberg (1961)
—»— C »	84 %	70–140 %	Egeberg (1961)
Fibrinogen	260 mg %	150–400 mg %	Jacobsson (1955), mod. by Blombäck & Blombäck (1956)

METHODS

Collection of blood. Venepuncture was performed with a siliconized needle, and the initial 2—3 ml of blood were discarded. Nine volumes of blood were then collected directly into a plastic tube containing one volume of 3.13 per cent w/v sodium citrate dihydrate. This was mixed by gentle inversion and centrifuged at 2,500 r.p.m. (ca. 1,800 G) for 30 minutes at 4° C. The plasma was removed with a siliconized pipette and stored in plastic tubes at —20° C. until assayed.

Assay of clotting factors. The plasma to be tested was thawed at 37° C., transferred to an icebath, and tested within 30 minutes. All reagents were kept in icebath, except for the calcium chloride and the calcium-proconvertin reagent mixture which were kept at 37° C., and the cephalin suspension which was kept at room temperature. All tests were carried out at 37° C., at least in duplicate. The clotting times were translated into per cent of normal activity by means of correlation graphs (*i. e.*, dilution curves of our human standard plasma). See Hjort, Rapaport & Owren (1955) for the dilution technique.

Antihemophilia B factor was assayed in a one-stage cephalin system (Egeberg 1961). The following mixture was incubated in new glass test tubes for 6 minutes: 0.2 ml cephalin suspension in optimal concentration, 0.2 ml test plasma diluted 1:10, and 0.2 ml plasma from a patient with severe hemophilia B. The mixture was then recalcified with 0.2 ml calcium chloride in optimal concentration, and the clotting time recorded.

Proconvertin was assayed in the system of Aas (1952): a mixture of 0.2 ml plasma from a patient with severe proconvertin deficiency, 0.2 ml human brain thromboplastin, and 0.2 ml test plasma diluted 1:10 was incubated for 3 minutes. It was then recalcified with 0.2 calcium chloride in optimal concentration, and the clotting time recorded.

Prothrombin was assayed in the system of Hjort *et al.* (1955) as modified by Hasselback & Hjort (1960). This system is specific for prothrombin. A mixture of 0.2 ml filtered, adsorbed ox plasma, 0.2 ml Russel viper venom in cephalin, and 0.2 ml test plasma diluted 1:50 was incubated for 3 minutes. It was then recalcified with 0.2 ml of a mixture containing 0.1 ml calcium chloride 70 mM and 0.1 ml proconvertin reagent, and the clotting time recorded.

EXPERIMENTAL PROCEDURE

The patient was kept in bed on regular hospital diet. Blood was collected for a control specimen, and he then received 150 mg. Warfarin intravenously. Twenty-four and 48 hours later, he reiceived additional doses of 100 mg. At intervals, blood was collected for assay of the clotting factors. The plasma samples were frozen at —20° C., and all assays carried out when the experiment was finished.

After 72 hours the experiment was terminated,

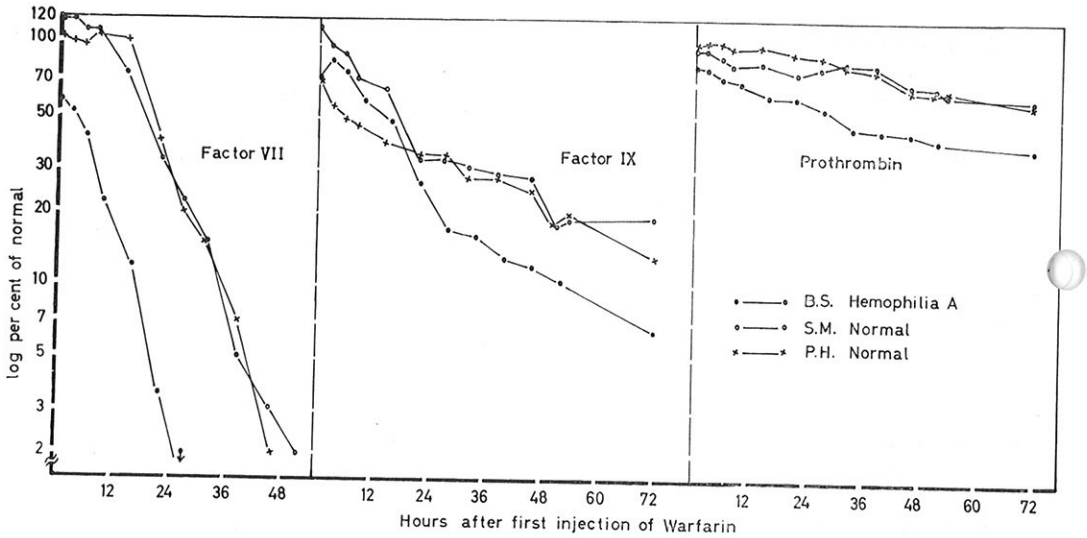

Fig. 1. The turnover of factor VII, factor IX and prothrombin in a patient with hemophilia A and in two healthy persons.

and he received 100 mg vitamin K_1 (Mephyton, Merck Sharp & Dohme, Rahway, N.J., U.S.A.) intravenously. He had no bleeding or untoward reactions during the experiment.

The same procedure was followed for the two normal persons, except that they were not kept in bed.

RESULTS

Fig. 1 shows the results.

Proconvertin remained nearly unchanged for a period of 4-14 hours, and then dropped exponentially. The T/2 can be derived graphically from the straight part of the curve: 300 minutes for the patient and 290 and 320 minutes for the two normal persons.

Antihemophilia B factor decreased progressively, but the T/2 cannot be accurately determined from the curves. The best estimate appears to be about 24 hours in all three persons.

Prothrombin also decreased progressively, but more slowly. The T/2 cannot be accurately determined, but appears to be about 3 days in all three persons.

DISCUSSION

Frick (1958) and Hasselback & Hjort (1960) have shown that the dose of Warfarin used in this experiment gives a maximum rate of fall of proconvertin and prothrombin in normal persons.

For proconvertin, the T/2-values agree with previous results in normals, *viz.*, 300—335 minutes (Hasselback & Hjort, 1960). Similar results were reported by Loeliger, Esch, Cleton & Booij (1959). The lag phase showed greater variations in this experiment (4—14 hours) than in the experiment (6—9 hours) reported by Hasselback & Hjort (1960).

For the antihemophilia B factor, the literature contains only few careful measurements. After a blocking dose of phenylindanedione, Stapp (1958) found its turnover rate closer to that of proconvertin than to that of prothrombin. Aggeler *et al.* (1956) and Bolton & Clarke (1959) tranfused hemophilia B patients, and found that factor IX survived for several days. Using an isotope technique in normals, Adelson, Rheingold, Parker, Steiner & Kirby (1960) arrived at a T/2 of 8.5 days.

For prothrombin, Borchgrevink, Egeberg, Pool, Skulason, Stormorken & Waaler (1959) arrived at a T/2 of 2½—3 days, based on a blocking experiment with penylindanedione in a normal individual and on a transfusion experiment in a patient with an isolated prothrombin deficiency. Hasselback & Hjort (1960) found about 2½ days after Warfarin, both in normals and in heparinized normals.

With this experimental technique, therefore, the turnover rate of these clotting factors was not prolonged in our patient with severe hemophilia. The data are conclusive for proconvertin; for antihemophilia B factor and for prothrombin some reservation is necessary since they have a slower turnover rate which makes it more difficult to interpret the curves.

Our findings support the conclusions of Hasselback & Hjort (1960) that the rapid turnover rate of clotting factors is not caused by a continuous subclinical *in vivo* coagulation. The present technique cannot rule out the possibility that a smaller fraction turns over as a consequence of coagulation, and our results do therefore not disprove the theory of a continuous coagulation process in normal individuals. However, the rapid turnover rate of clotting factors should not be used as evidence for such a process.

SUMMARY

The disappearance of prothrombin, proconvertin (factor VII) and antihemophilia B factor (factor IX) from plasma after blocking of the liver synthesis by large doses of Warfarin reflects the *in vivo* turnover of these factors. By this technique, the turnover was investigated in a patient with severe hemophilia A and in two healthy persons.

The turnover of proconvertin was normal in the patient. It was probably also normal for prothrombin and the antihemophilia B factor, but these data are less conclusive. These observations give additional support to the view that the rapid turnover of these factors is not caused by continuous *in vivo* coagulation.

REFERENCES

Aas, K.: Prokonvertin og konvertin. Thesis. Oslo, Akad. Trykningssentral. 1952, 90 pp.

Adelson, E., Rheingold, J. J., Parker, O., Steiner, M. & Kirby, J. C.: Survival of plasma thromboplastin component and antihemophilic globulin in normal humans. Clin. Research *8*, 369, 1960.

Aggeler, P. M., Fowell, A. H., Johnson, F. F., Hoag, S. & Pool, J. G.: Intravenous use of a concentrated plasma P.T.C. preparation. Proc. VI Int. Congr. Haemat. Boston 1956, p. 490. Grune & Stratton, New York 1958.

Blombäck, B. & Blombäck, M.: Purification of human and bovine fibrinogen. Arch. Kemi *10*, 415, 1956.

Bolton, F. G. & Clarke, J. E.: A method of assaying Christmas factor; its application to the study of Christmas disease (Factor-IX deficiency). Brit. J. Haemat. *5*, 396, 1959.

Borchgrevink, C. F., Egeberg, O., Pool, J. G., Skulason, T., Stormorken, H. & Waaler, B.: A study of a case of congenital hypoprothrombinaemia. Brit. J. Haemat. *5,* 294, 1959.

Egeberg, O.: Assay of antihemophilic A, B and C factors by one-stage cephalin systems. Scandinav. J. Clin. & Lab. Investigation *13,* 140, 1961.

Frick, P. G.: Studies on the turnover of stable prothrombin conversion factor in man. Acta Haemat. *19,* 20, 1958.

Hasselback, R. & Hjort, P. F.: Effect of heparin on in vivo turnover of clotting factors. J. appl. Physiol. *15,* 945, 1960.

Hjort, P. F.: Intermediate reactions in the coagulation of blood with tissue thromboplastin. Scandinav. J. Clin. & Lab. Investigation *9,* Suppl. *27,* 1957.

— Rapaport, S. I. & Owren, P. A.: A simple, specific one-stage prothrombin assay using Russell's viper venom in cephalin suspension. J. Lab. & Clin. Med. *46,* 89, 1955.

— & Stormorken, H.: A study of the in vitro and in vivo effects of a synthetic heparin-like anticoagulant: dextran sulphate. Scandinav. J. Clin. & Lab. Investigation *9,* Suppl. 29, 1957.

Jacobsson, K.: Studies on the determination of fibrinogen in human blood plasma. Scandinav. J. Clin. & Lab. Investigation *7,* Suppl. 14, 1955.

Loeliger, E. A., Esch, B. van der, Cleton, F. J. Booij, H. L.: On the metabolism of the coagulation factor VII. VI Europ. Congr. Haemat., London 1959, P. 223 in program.

Owren, P. A.: The coagulation of blood. Acta med. Scandinav. *128,* Suppl. 194, 1947.

Nygaard, K. K.: A direct method of counting platelets in oxalated plasma. Proc. Staff. Meet. Mayo Clin. *8,* 365, 1933.

Stapp, W. F.: A one stage method for the assay of antihemophilic factor B (AHF B). Scandinav. J. Clin. & Lab. Investigation *10,* 169, 1958.