EVIDENCE FOR A NEW COAGULATION FACTOR

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1. Introduction

Fibrin-stabilizing factor (FSF; factor XIII) is a calcium-dependent enzyme, probably present in plasma as an inactive precursor; on activation it is responsible for the conversion of soluble to insoluble fibrin during the terminal stage of blood coagulation.

While investigating factors affecting the rate of fibrin stabilization it was observed that this occurred more rapidly if the plasma and fibrinogen solution were in contact prior to clotting by a calcium-thrombin solution. This was further investigated as follows.

2. Methods and results

Pooled citrated plasma in equal quantities from 10 normal subjects diluted to twice its volume with tris buffer (pH 7.4; ionic strength 0.15) was used as a source of FSF. Human fibrinogen (Lister Institute) was made up to a concentration of 1.0 mg/ml in tris buffer (pH 7.4; ionic strength 0.15) and the solution shown to be free of FSF activity before use by clotting 1.0 ml (25 units) of a fresh solution of thrombin (S.Maw, Son and Sons Ltd.) in 1% calcium chloride, allowing 30 min for stabilization, and demonstrating immediate solubility of this clot in 2.0 ml of 2% monochloroacetic acid.

The following mixture was prepared (ml):

Pooled normal plasma	0.3
Saline (0.9%)	0.2
Fibrinogen (1.0 mg/ml)	1.0

and was clotted by 1 ml of a fresh solution of calcium-thrombin (25 units/ml in 1% calcium chloride) immediately after the fibrinogen solution had been added to the plasma and saline. An identical mixture was preincubated for 2 hr, and on another occasion for 3 hr, and then clotted by the addition of 1.0 ml calcium-thrombin. The rate of conversion of soluble to insoluble fibrin was measured as previously described [1]. Controls using saline instead of plasma showed no insoluble fibrin formed. All assays were performed in duplicate.

The results are shown in table 1. It can be seen that fibrin stabilization is more rapid if fibrinogen solution is incubated with plasma prior to clotting.

A posssible explanation of this phenomenon is that for optimal FSF activity, fibrinogen and FSF need time to react together in some way prior to clotting. This possibility was investigated as follows.

The rate of insoluble clot formation was determined using the following clotting mixtures (ml):

(a) Pooled normal plasma	0.3
Saline (0.9%)	0.3
Fibrinogen (1.0 mg/ml)	1.0
(b) Pooled normal plasma	0.3
Plasma from a patient with congenital deficiency of FSF	0.3
Fibrinogen (1.0) mg/ml)	1.0

In (b) the FSF-free plasma and fibrinogen were mixed 3 hr before their addition to the pooled normal plasma.

1.0 ml of calcium-thrombin was added to both (a) and (b) *immediately* after the addition of the fibrinogen or fibrinogen–FSF-free plasma mixture to the pooled normal plasma. Thus the fibrinogen was not allowed to incubate with normal plasma prior to clotting but in (b) it had incubated with FSF-free plasma prior to clotting.

Table 1
Effect on the rate of fibrin stabilization of prior incubation of fibrinogen with normal plasma prior to clotting.

Source of fibrinogen	Incubation time of fibrinogen with plasma (hr)	Rate of fibrin stabilization (mg/min)
Lister Institute (human)	2	0.264
	negligible	0.125
Lister Institute (human)	3	0.306
	negligible	0.100
Kabi, Grade L (human, lyophilized)	1	0.577
	negligible	0.170
Kabi, Grade B_1 (bovine, lyophilized)	1	0.134
	negligible	zero

The rate of fibrin stabilization of (a) was 0.105 mg/min and of (b) was 0.817 mg/min.

Thus incubation of fibrinogen with FSF-free plasma prior to clotting resulted in more rapid fibrin stabilization and this rules out the suggested explanation of an association between FSF and fibrinogen. Mixture (b) contains slightly more fibrinogen than mixture (a) due to that present in the FSF-free plasma. This would produce slightly more rapid stabilization of fibrin but would certainly not account for the difference since addition of this amount of fibrinogen (0.1 mg) would cause an increase of only about 0.03 mg/min [1].

A further possible explanation is that the low-temperature, ether-fraction technique, used to prepare the purified fibrinogen might have caused a biochemical or biophysical change modifying the ability of the fibrinogen to form stabilized fibrin, and that this could be reversed by re-exposure to plasma. Thus fibrinogen (Kabi) prepared by ethanol fractionation in the presence of glycine at −3% [2] was subjected to the same test.

Solutions (1.0 mg/ml) of fibrinogen (Kabi) grade L (human, lyophilized) and grade B_1 (bovine, lyophilized) in tris buffer (pH 7.4; ionic strength 0.15) were incubated in a water bath at 40° for 3 hr to destroy any contaminating FSF. The absence of FSF was confirmed as described above. Each FSF-free fibrinogen preparation was then assayed as described above to determine the effect of prior incubation with normal plasma on the rate of fibrin stabilization.

The results are shown in table 1. The two preparations of fibrinogen Kabi showed the same phenomenon as the fibrinogen supplied by the Lister Institute in that stabilization of fibrin is more rapid after prior incubation of fibrinogen with normal plasma. The magnitude of the effect of preincubation appears different for the different sources of fibrinogen. This might well reflect changes in the fibrinogen produced by the method of preparation since the rate of stabilization without preincubation is different for the different types of fibrinogen.

3. Conclusions

These results suggest the existence of a hitherto undescribed plasma factor which modifies in such a way as to prepare it for subsequent rapid stabilization when it is converted to fibrin. This occurred with two different preparations of human fibrinogen and also with one preparation of bovine fibrinogen and it therefore seems unlikely that this property is induced during the process of preparation, but even if this proves to be the case the existence of a hitherto unknown plasma factor acting on the fibrinogen must still be inferred.

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References

[1] W.D. Walls and M.S.Losowsky, Thromb. Diath. Haemorrhag. 21 (1969) 134.
[2] B.Blomback and M.Blomback, Arkiv. Kemi. 10 (1956) 415.