Inheritance of Deficiency of Fibrin-stabilizing Factor (Factor XIII)

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Fibrin-stabilizing factor (FSF or Factor XIII) is a plasma protein which, during the course of blood coagulation, is hydrolytically converted by thrombin into a transpeptidating enzyme, "fibrinoligase." This enzyme catalyzes the selective formation of γ-glutamyl-ϵ-lysine bridges between fibrin units leading to the production of more stable structures, which unlike simple fibrin, can no longer be dispersed in 1% monochloroacetic acid or in 5 M urea (Lorand, Downey, et al. 1968; Lorand, Rule, et al. 1968). Patients with FSF deficiency have a tendency to bleed and show poor wound healing after surgery or dental extraction. In such persons, the usual tests for clotting give normal values except for a considerable degree of crumbling of the clot and a striking diminution of the amplitude of the thromboelastogram. The diagnosis is confirmed by a rapid dispersion of the clot obtained by recalcification of the plasma in a 5M urea or a 1% monochloroacetic acid solution. The FSF deficiency has been described in 44 individuals (27 males and 17 females) from 27 families (table 1).

Ratnoff and Steinberg (1968) have postulated that this disorder may be inherited in two ways: autosomal recessive and X-linked recessive. This hypothesis is based on the preponderance of male patients and the high frequency of consanguinity in families where female members are affected (table 1A) and the relative infrequency of consanguinity in families where only male patients are affected (table 1B). Definitive evidence for this concept would be provided by the demonstration of reduced FSF activity in mothers but not in fathers of some families with FSF deficiency where only males were affected.

Recently, Lorand et al. (1969) have shown that the fibrin-stabilizing factor, after activation by thrombin, is able to catalyze the incorporation of the fluorescent amine, monodansylcadaverine [or N-(5-aminopentyl)-5-dimethylamino-1-napthalenesulfonamide], into casein as shown in figure 1. The reaction provided the basis of a sensitive fluorimetric method for measuring the activity of the enzyme. The present communi-

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cation describes the investigation of seven families with FSF deficiency using this assay system.

MATERIALS AND METHODS

For these studies, approximately 8 ml of venous blood is collected in tubes containing 2 ml of ACD (2.5 g sodium citrate dihydrate, 1.25 g citric acid, and 2.0 g glucose in 100 ml water). The plasma is separated by centrifugation at 2,500 RPM for 20 min. The decanted fresh plasma is frozen and stored at −20° to −70° C.

TABLE 1

SUMMARY OF DATA ON 27 FAMILIES WITH FSF (FACTOR XIII) DEFICIENCY

AUTHORS	ORIGIN	AFFECTED MEMBERS		CONSANGUINITY
		M	F	
	A. Families with Affected Females			
1. Duckert et al. 1961 and subsequent papers	Switzerland	2	1	+
		0	3	+
		2	0	+
2. Mandelli 1963	Italy	0	2	+
3. Masure 1963	Belgium	1	1	.....
4. Ikkala et al. 1964	Finland	0	1	+
5. Ikkala et al. 1964	Finland	1	1	.....
6. Fisher et al. 1966	Morocco	0	1	+
7. Fischer and Lechner 1968	Austria	1	1	+
8. Egeberg 1968 Case 1	Norway	0	1	.....
9. Egeberg 1968 Case 2	Norway	0	1	.....
10. Zahir 1969	Pakistan	0	1	+
11. Leiba et al. 1969	Israel	3	0	+
		0	1	+
12. deKiewiet and Nossel 1969	Cuba	0	1	+
13. Fradera et al. 1969 (Family D)	Puerto Rico	0	1	+
	B. Families with Affected Males			
14. Ikkala and Nevanlinna 1962	Finland	2	0	.....
15. Josso et al. 1964	France	1	0	.....
16. Josso et al. 1964	France	1	0	+
17. Barry and Delâge 1965	French Canadian	2	0	.....
18. Amris and Ranek 1965	Denmark	1	0	.....
19. Tsevrenis et al. 1965	Greece	1	0	.....
20. Losowsky et al. 1965 Case 1	England	2	0	.....
21. Losowsky et al. 1965 Case 2	England	1	0	.....
22. Losowsky et al. 1965 Case 3	England	1	0	.....
23. Britten 1967	USA	1	0	.....
24. Bouhasin and Altay 1968	USA	1	0	.....
25. Newcomb et al. 1968	USA	1	0	.....
26. Fukutake 1969, personal communication	Japan	1	0	.....
27. Fradera et al. 1969 (Family E)	Puerto Rico	1	0	.....
	Total			
	.....	27	17	.....

The assay for FSF was carried out according to the procedure described by Lorand et al. (1969): 0.2 ml of citrated plasma is mixed with 0.05 ml of 50% (v/v) aqueous glycerol in a 15 × 100 mm (Sorvall pyrex) test tube. The mixture is brought rapidly to 56° C and kept at this temperature for two and a half minutes. It is then immersed in ice and allowed to cool to room temperature (24° C). Into this mixture is added 0.05 ml of 0.2 M glutathione solution [dissolved in 50% (v/v) aqueous glycerol and adjusted to *p*H 7.5 with 3N NaOH].

Activation of FSF is initiated by adding 0.2 ml of thrombin [125 NIH units/milliliter dissolved in 25% (v/v) aqueous glycerol and 0.02 M $CaCl_2$ at *p*H 7.5] and

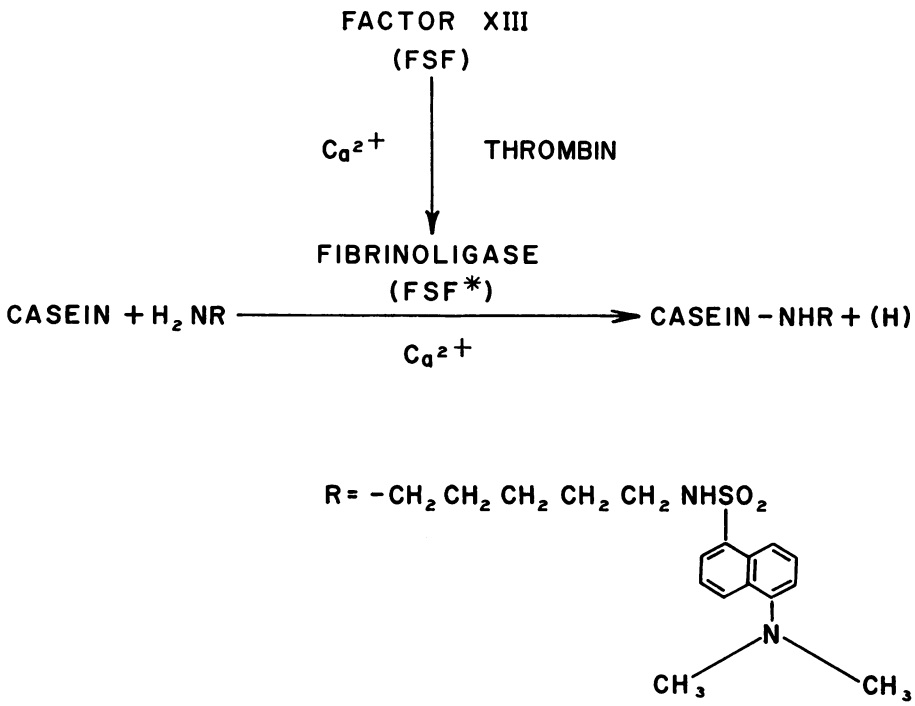

FIG. 1.—A coupled system for measuring the concentration of FSF in terms of incorporation of a labeled amine (fluorescent monodansylcadaverine) into an acceptor protein (casein). The factor (FSF) is converted to the active transpeptidase (FSF* or fibrinoligase) by the addition of thrombin.

is allowed to proceed for 20 min. To this mixture is added 0.5 ml of the synthetic amine substrate (2 mM monodansylcadaverine solution in a 0.05 M tris-HCl at *p*H 7.5) and 3 mM $CaCl_2$. The incorporation reaction is started with the addition of 1.0 ml of 0.4% casein solution, made up in a mixture containing 10% glycerol, 3 mM $CaCl_2$, and 0.05 M tris-HCl at *p*H 7.5. After dialysis the solution is clarified by centrifugation at 20,000 RPM for 60 min in a Spinco L centrifuge using a No. 30 rotor. Amine incorporation is stopped 30 min later by adding 2 ml of 10% trichloroacetic acid. The protein precipitate is washed successively with 6 × 10 ml of ethanol-ether (1:1) and dried. Finally, it is taken up in a 2-ml solution of 8 M urea and 0.5% sodium dodecylsulfate in 0.05 M tris-HCl at *p*H 8, for measuring the protein-bound amine.

Fluorescence intensities were obtained in an Aminco-Bowman spectrophotofluorimeter (excitation at approximately 355 mμ; emission at approximately 525 mμ) using monodansylcadaverine at a concentration of 1 μmole/liter in the same solvent system as a reference standard. Units of FSF are expressed as defined in Lorand et al. (1969), and duplicate measurements on the same sample show variations no greater than one unit per ml.

The seven families studied were as follows: Family A (Britten 1967), Family B (Fukutake 1969, personal communication), Family C (deKiewiet and Nossel 1969; Lorand et al. 1969), Family D (Fradera et al. 1969, Case 1), Family E (Fradera et al. 1969, Case 2), Family F (Barry and Delâge 1965), and Family G (Newcomb et al. 1968). These data were compared with a group of normal controls among male and female laboratory personnel.

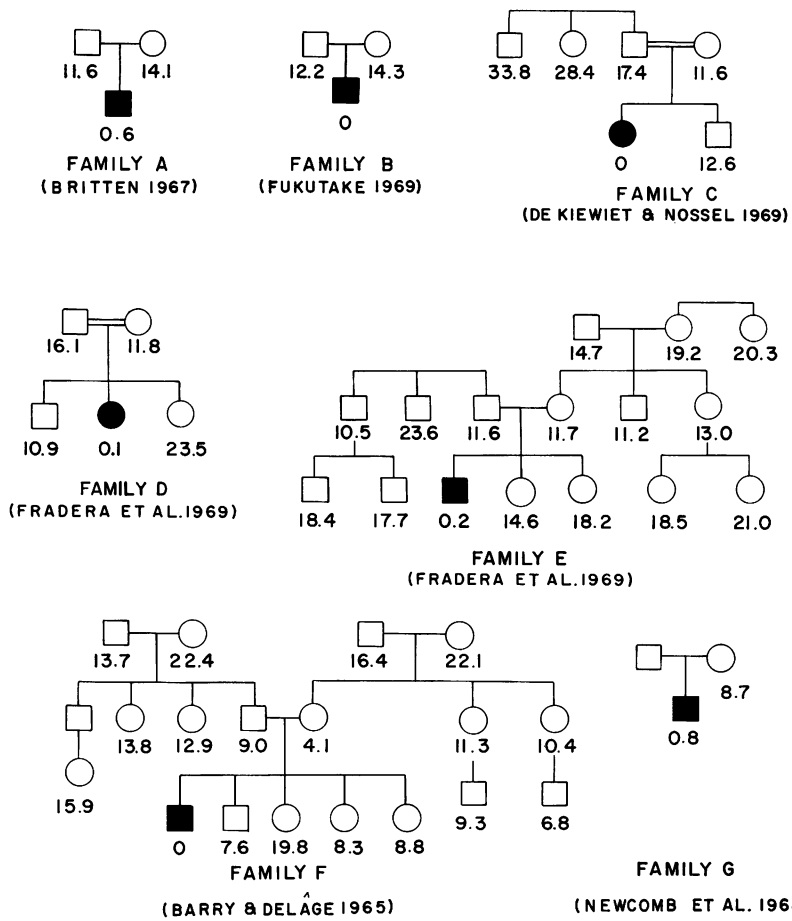

FIG. 2.—FSF activity expressed as units/milliliter in pertinent members of seven families with FSF deficiency.

RESULTS AND DISCUSSION

The data from studies on the families are tabulated in figure 2 and summarized in figure 3. The mean and standard deviation for 72 controls was 19.0 ± 5.5 units/milliliter; for 13 heterozygotes (parents of patients), 11.8 ± 3.3 units/milliliter; and for seven homozygotes (patients), 0.2 ± 0.1 units/milliliter. There was no essential difference in the FSF activity between males and females among the controls, heterozygotes, and homozygotes. While there was an appreciable overlap between the controls and heterozygotes making it difficult to classify a specific individual, the values for the heterozygotes as a group were significantly lower than those for the controls as a group ($P < .001$).

In examining individual pedigrees, five out of the seven families showed only affected males. Of these, Families A, B, E, and F yielded useful information on both

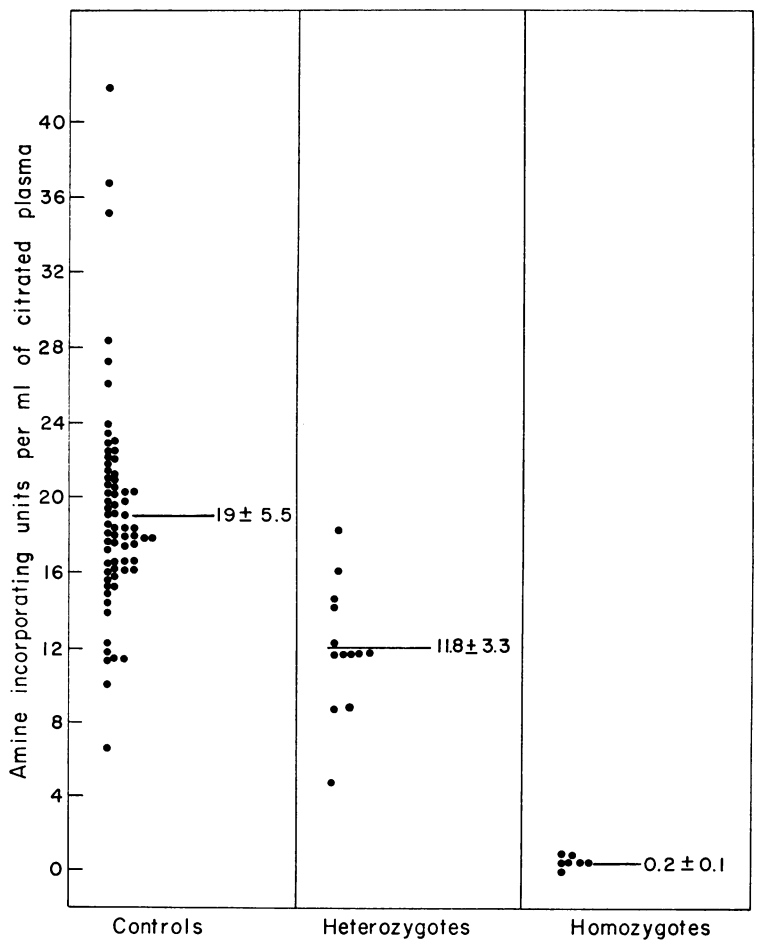

FIG. 3.—Summary of data on controls, heterozygotes, and homozygotes

parents and in each instance, the FSF value in the father was in the same range as that of the mother and was significantly lower than the range seen in normal controls. In Family G, the mother showed reduced values, although no data are available on the father. The FSF values for the fathers and mothers in Families C and D with an affected female were similar to the values seen in Families A, B, E, and F with an affected male. It is always possible that in our investigation of five of the 14 families with FSF deficiency where only males are affected, we encountered only the ones transmitted by an autosomal recessive mode of inheritance. However, in these families, at least, there is no biochemical evidence to support the hypothesis proposed by Ratnoff and Steinberg (1968).

SUMMARY

A sensitive fluorimetric method measuring the incorporation of monodansylcadaverine into casein has been developed for the direct measurement of plasma FSF activity expressed as units/milliliter. Using this method, the levels of FSF activity among parents of children affected with total deficiency were significantly lower than among comparable normal controls. The FSF levels were equally decreased for both fathers and mothers in families with only affected males and in families with affected females. These findings support the concept that congenital FSF (Factor XIII) deficiency is transmitted as an autosomal recessive trait.

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