

Fibrin/Fibrinogen Degradation Products in Sera of Normal Infants and Children

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The phenomenon of disseminated intravascular coagulation is becoming increasingly recognized as an important factor in the pathophysiology of many disease states including the haemolytic uraemic syndrome (Piel and Phibbs, 1966); meningococcaemia with collapse (McGehee, Rapaport, and Hjort, 1967); purpura fulminans (Taylor and Wright, 1956; Little, 1959); *Esch. coli* diarrhoea (McKay and Wahle, 1955); and possibly some cases of haemorrhagic disease of the newborn (Wardle, 1968). Furthermore, painstaking work by Boyd (1967) has shown fibrin deposition to have occurred in a series of neonatal deaths similar to that found in disseminated intravascular coagulation, and this was considered to be an important contribution to the cause of death. Finally, Stark, Abramson, and Erkan (1968) have suggested that disseminated intravascular coagulation is the underlying cause of hyaline membrane disease, citing among others the evidence of Ambrus *et al.* (1963) that the fibrinolytic enzyme system may be impaired in premature babies. Some of these conditions may represent the clinical manifestations of the Shwartzman reaction, and the topic has been the subject of several recent reviews (McKay, 1965; McKay and Müller-Berghaus, 1967).

During the proteolysis of fibrinogen and fibrin by the fibrinolytic enzyme plasmin, several fragments are released that are unclottable and incapable of further digestion (Nussenzweig and Seligmann, 1960; Alkjaersig, Fletcher, and Sherry, 1962). Two of these fibrinogen/fibrin degradation products (FDP), the so-called D and E fragments, have an antigenic determinant which is identical to the parent fibrinogen. This property has made it possible to adapt the tanned red cell haemagglutination inhibition immunoassay of Boyden (1951) for the quantitative estimation of serum FDP (Murakami, Sekimoto, and Yasuda, 1965; Merskey, Kleiner, and Johnson, 1966), and thus provide some inform-

ation on the level of actual *in vivo* fibrin deposition and lysis in those clinical states not associated with fibrinogenolysis. The sensitivity of this technique is such that it is possible to detect abnormally high values of serum FDP before there is clear collateral evidence of a consumption coagulopathy in terms of thrombocytopenia, hypofibrinogenaemia, and depleted coagulation factors. Thus, the immunoassay may be used to show the presence of occult intravascular coagulation and fibrinolysis. It is apparent that any parameter that gives an index of the presence or degree of intravascular coagulation will have great importance, particularly if it can also be used as an early diagnostic tool, and to follow progress in the event of treatment which, in the instances given, might well be heparin.

The purpose of the following investigation was to demonstrate and define the levels of the circulating FDP in normal infancy and childhood before the investigation of those pathological states believed to be associated with focal or disseminated intravascular coagulation and fibrinolysis.

Methods

Studies were made of 112 apparently healthy children who had suffered no recent infections and who were receiving no form of medication. They included functional enuretics, children for adoption, and children about to undergo surgical treatment such as circumcision and herniorrhaphy. Blood was obtained by clean venepuncture without constriction when it was required for other routine investigations. Included among this number were 26 babies from the newborn nurseries whose mothers agreed to their being venepunctured. A proportionately larger number of infants and young children was selected. 2.5 ml. blood were added to a non-siliconized test-tube containing 0.05 ml. aprotinin (5000 units/ml.) and placed in a water bath of 37 °C. for 4 to 24 hours. The serum was removed after centrifugation at 2000 r.p.m. for 10 minutes, and one-tenth of its volume of thrombin (100 units/ml.) was added and incubated for 30 minutes at 37 °C. to remove residual fibrinogen. FDP assay was performed by the method of Merskey (Merskey

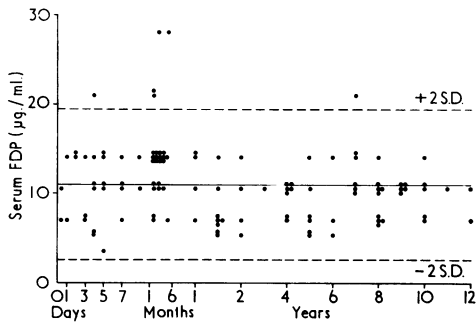


FIG. 1.—Fibrin/fibrinogen degradation products in the sera of 112 normal infants and children. Mean level $10.9 \pm 4.3 \mu\text{g./ml.}$

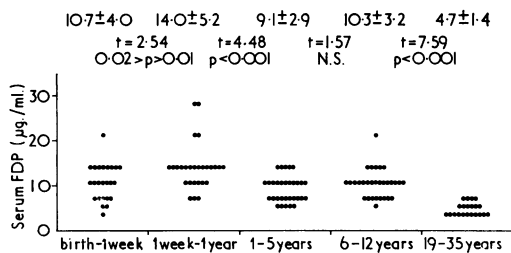


FIG. 2.—Serum levels of FDP found in different age-groups.

et al., 1966), as previously described by Das *et al.* (1967) and Woodfield *et al.* (1968). Each sample was code numbered and those responsible for the immunoassay were unaware of the clinical details. Moreover, the 24 samples selected for each assay run were chosen at random, thus avoiding the possibility of assaying each age-group as an isolated batch.

Further blood was available in 91 of these children

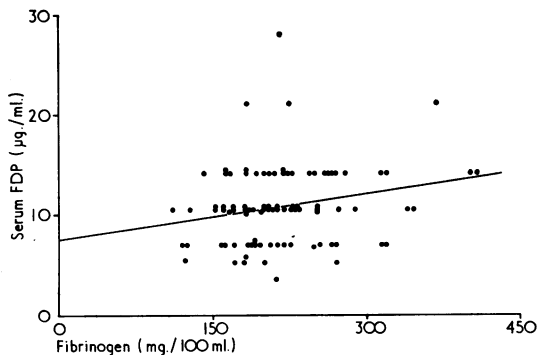


FIG. 3.—Correlation between serum FDP and plasma fibrinogen in 91 infants and children. Regression equation $y = 7.64 + 0.146x$. Coefficient of correlation $r = 0.22$; $0.05 > p > 0.02$.

allowing fibrinogen assays to be done on the same specimens. These were performed on aprotinin citrate plasma samples and measured by the method of Ellis and Stransky (1961).

Results

Normal childhood levels of FDP. The over-all figures of 112 children gave a mean serum FDP level of $10.9 \pm 4.3 \mu\text{g./ml.}$ There was no statistical difference between males (63 children $10.3 \pm 4.1 \mu\text{g./ml.}$) and females (49 children $11.7 \pm 4.4 \mu\text{g./ml.}$).

Breakdown of these children by age-groups shows a small but significant difference between babies in the first week of life ($10.6 \pm 4.0 \mu\text{g./ml.}$) and those aged between 1 week and 1 year ($14.0 \pm 5.2 \mu\text{g./ml.}$), as shown in the scattergram (Fig. 1); and there was a small but again statistically significant fall after 1 year. Further statistical analysis taking groups aged 2 months to 1 year and 1 year to 2 years confirmed the fall to be occurring at about the 1-year level. A group of 21 young adults aged from 19 to 35 years, in which serum FDP levels were assayed at the same time, had significantly lower levels ($4.7 \pm 1.4 \mu\text{g./ml.}$) when compared with both the over-all childhood population and the 6- to 12-year-old age-group.

The over-all plot of FDP against age is shown in Fig. 2.

Plasma fibrinogen. Plasma levels obtained gave a value of $217 \pm 59 \text{ mg./100 ml.}$ over the whole group. When split for ages as previously, the only significant difference between groups was obtained between the first-week babies and the 1-week to 1-year group ($201 \pm 45 \text{ mg./100 ml.}$ —15 cases and $245 \pm 57 \text{ mg./100 ml.}$ —17 cases, respectively; $t = 2.3816$; $0.05 > p > 0.02$).

The correlation of serum FDP against plasma fibrinogen is shown in Fig. 3 and is just statistically significant; $r = 0.2200(1/\sqrt{N-1}) = 0.105$; $0.05 > p > 0.02$, the regression equation being $y = 7.64 + 0.146x$.

Discussion

Though normal ranges of serum FDP are now available for adults (Das *et al.* 1967) and pregnant women (Woodfield *et al.* 1968), no comparable data exist for children. Bonifaci, Baggio, and Gravina (1968), using the relatively insensitive and semiquantitative technique of immunoelectrophoresis, were able to show the presence of FDP in 7 out of 15 normal newborns at the twelfth hour, but were unable to detect them thereafter. More recently Stiehm and Clatanoff (1969) have

shown FDP to be present in the sera of 65% of 208 term vaginally delivered infants using a semi-quantitative modification of the tube precipitin method of Merskey *et al.* (1966). Again they were no longer able to show the presence of FDP after the first 24 hours. When using the tanned red cell haemagglutination inhibition immunoassay technique Das *et al.* (1967) were able to show the presence of circulating FDP in 95% of normal adults with a sensitivity of as little as 0.6 $\mu\text{g./ml.}$ The present investigation using the same method shows that serum FDP levels are detectable in all the children studied and that indeed the mean level is somewhat greater than in adult life.

The reasons for this are unknown, though it is interesting to speculate that growth may be a factor, particularly as the highest levels were found in the 1-week to 1-year age-group where growth is particularly rapid. Similarly it has been found that the levels of serum FDP rise in the last trimester of pregnancy (Woodfield *et al.*, 1968), again a period of increased growth; but here there are probably other important factors operating. Experimental support for this view exists in the work of Astrup (1968) who has reviewed the role of fibrin formation and fibrin resolution in tissue growth and tissue repair. He has pointed out that in tissue repair fibrin is the scaffold for migrating cells, and that during fibroblastic proliferation and capillary growth the fibrin is removed.

The source and ultimate significance of the findings of serum FDP in normal children remain obscure. Astrup (1956) has suggested that there is continuous deposition of fibrin throughout the vasculature and its subsequent removal by fibrinolytic enzymes. Circulating FDP therefore may be a result of this phenomenon, but on the other hand the extravascular space is known to contain possibly up to 50% of total body fibrinogen (Hammond and Verel, 1959), and Benz (1968) has also shown the presence there of both plasminogen activator and FDP. The possibility of an extravascular contribution to the serum FDP representing byproducts of tissue growth, damage, and repair must be considered.

Because of the pathophysiological importance of the finding of FDP in normal serum, it must be certain that they do not represent an *in vitro* breakdown of fibrin or the presence of residual fibrinogen in the assay system. The efficacy of aprotinin, however, in the total prevention of fibrinolysis is generally accepted as shown, for example, in the work of Dubber *et al.* (1968). Thrombin is added to the assay system in excess, and Das (1969) has shown by immunoelectrophoresis that no

fibrinogen remains detectable in the liquor after the production of the fibrin clot. Das has also shown that the fibrinopeptides released during the clotting of purified fibrinogen by thrombin in the presence of aprotinin do not produce haemagglutination inhibition. Previous studies in adults (Das *et al.*, 1967; Woodfield *et al.*, 1968) have failed to show a correlation between serum FDP and plasma fibrinogen, and thus the absolute significance of the correlation found in the present investigation is unknown. The correlation achieved was only marginally significant at the 0.05 level, and when Chauvenet's criteria were applied to the fibrinogen figures reducing them to a statistically 'normal' population, the FDP/fibrinogen correlation was no longer statistically significant ($r = 0.1065 (1/\sqrt{N-1}) = 0.1067$).

The absolute levels of plasma fibrinogen obtained compare with previously recorded normal limits (Aballi and De Lamerens, 1962) showing normal values at all age-groups, and again provide a baseline for further studies employing the Ellis and Stransky method.

The excessive amounts of FDP found in pathological conditions possess in themselves anticoagulant properties inhibiting platelet aggregation and the thrombin fibrinogen reaction (Kowalski *et al.*, 1964), thromboplastin formation (Niewiarowski, Latallo, and Stachurska, 1959), and fibrin polymerization (Alkjaersig *et al.*, 1962), thus adding to the bleeding diathesis. The finding of significantly raised levels of serum FDP provide incontrovertible evidence of either primary or secondary fibrinolysis, and the highest levels have generally been discovered in defibrination syndromes, such as in the case of Merskey *et al.* (1966) when a serum FDP level of 768 $\mu\text{g./ml.}$ was recorded.

Summary

Fibrin/fibrinogen degradation products have been shown in the sera of 112 healthy infants and children of all ages, and the normal levels defined ($10.9 \pm 4.3 \mu\text{g./ml.}$). These are significantly greater than those found in adults, and will provide baseline data for further studies involving the measurement of serum FDP in a variety of defined conditions in which intravascular coagulation is thought to occur.

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