

Dysfibrinogenaemia and liver cell growth

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SUMMARY From the evidence presented, it is proposed that 'dysfibrinogenaemia' represents the production of normal fetal fibrinogen by rapidly proliferating liver cells in both regenerating and neoplastic tissue. Prolongation of the reptilase clotting time, which was formerly believed to reflect dysfibrinogenaemia, may be rather the result of hepatocytic death.

In the context of these studies, dysfibrinogenaemia (DF) is defined as an excess of immunoassayable fibrinogen, of 1 g per litre or more, over thrombin-clottable protein in the plasma. On this basis, DF was shown to be a reliable biological marker of clinically overt primary hepatocellular carcinoma (PLC) in adult black Africans (Barr *et al.*, 1976). Furthermore, this finding was associated with a prolonged reptilase clotting time (RT) in these patients.

Against this background, a study in Scotland was initiated in an attempt to detect preclinical PLC in adult patients with chronic liver disease, which, for this purpose, was defined as the persistence of any evidence of hepatic disorder for longer than six months. These patients were classified histologically as chronic alcoholic liver disease, chronic active hepatitis, haemochromatosis, or primary biliary cirrhosis.

Material and methods

Plasma samples were prepared as previously described (Barr *et al.*, 1976). Reptilase clotting times were performed by the method of Gralnick *et al.* (1971) with one modification; each vial of reagent containing 20 Batroxobin units (approximately 50 µg of enzyme) was reconstituted with 1.5 ml rather than 1.0 ml of distilled water.

Thrombin-clottable fibrinogen was assayed by the method of Ogston and Ogston (1966) and heat-precipitable fibrinogen by the method of Millar *et al.* (1971). In the latter method stored plasma samples were mixed with washed homologous group O red cells to give a haematocrit of approximately 50%.

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Immunoassayable fibrinogen was determined by a radial immunodiffusion technique based on that described by Fahey and McKelvey (1965). Antihuman fibrinogen antiserum was incorporated in the agar gel, and multiple aliquots of a human plasma protein solution were applied to appropriate wells for the construction of a standard graph for each plate.

An identical method was used for the estimation of serum alpha-fetoprotein, using serial dilutions of a human alpha-fetoprotein preparation for standardisation. Behring diagnostic reagents for these immunoassays were obtained from Hoechst Pharmaceuticals, Hounslow, Middlesex.

Serum fibrin-fibrinogen degradation products were measured by a standard tanned red cell haemagglutination-inhibition technique (Wellcome FDP kit).

All estimates were performed in duplicate and the mean result was recorded in each instance.

Results

Control data were obtained from 20 healthy colleagues in Aberdeen (Table 1). We then examined samples from eight Scottish patients with PLC. Of these, seven revealed a prolonged RT, but DF was detectable in none.

Table 1 Control data (median \pm 2 SD)

| | |
|--|-----------------|
| Reptilase clotting time(s) | 22.0 \pm 2.4 |
| Normal range | 19-25 |
| Thrombin-clottable fibrinogen (g/l) | 2.70 \pm 1.10 |
| Normal range | 1.5-4.0 |
| Heat-precipitable fibrinogen (ml/100 ml) | 2.94 \pm 1.52 |
| Normal range | 1.5-4.5 |
| Immunoassayable fibrinogen (g/l) | 2.80 \pm 1.09 |
| Normal range | 1.5-4.0 |

Results from patients with chronic liver disease were grouped according to four patterns (Table 2).

Table 2 *Patients with chronic liver disease*

| | Group | | | |
|---------|-------|----|----|----|
| | A | B | C | D |
| Samples | 22 | 18 | 14 | 21 |

A = dysfibrinogenaemia and prolonged reptilase time;
 B = prolonged reptilase time alone; C = dysfibrinogenaemia alone;
 D = normal reptilase time and no dysfibrinogenaemia.

The combined abnormality (DF plus prolonged RT) was demonstrable in as many samples as those on which the results were normal; and almost as many showed DF alone as prolonged RT alone. Hence DF and prolonged RT do not appear to reflect the same phenomenon, and both occurred too frequently to be indicative of the presence of PLC. In no patient could the prolonged RT be explained by hypofibrinogenaemia or raised levels of fibrin-fibrinogen degradation products.

In a further group of 10 patients with carcinoma metastatic to the liver, only one had DF and a prolonged RT demonstrable. This exceptional patient was an alcoholic. These findings further support the specific association of acquired DF with primary hepatic disease, which is predictable since fibrinogen is produced selectively in the liver (Straub, 1963).

Discussion

Abnormal fibrin monomer polymerisation has been described in 50% of a large series of patients with chronic parenchymal liver disease (Green *et al.*, 1976). This abnormality does not seem to be due to the presence of inhibitory activity (Green *et al.*, 1976), factor XIII deficiency (Lane *et al.*, 1977), or a major abnormality of the molecular structure of fibrin monomers (Lane *et al.*, 1977). The incidence of the defect is essentially identical with that obtained for DF in chronic liver disease in the present study (Table 1). Moreover, DF (as defined by us) and defective fibrin monomer polymerisation have been described in the same PLC patient (Verhaeghe *et al.*, 1972). This suggests that DF and abnormal fibrin monomer polymerisation in liver disease represent the same basic defect.

Gralnick *et al.* (1975) have reported 'defective' fibrin monomer polymerisation with normal human fetal fibrinogen. Since this finding is also the commonest abnormality in congenital dysfibrinogenaemia (Gralnick and Finlayson, 1972), it suggests that this latter entity may rather be due to a persistence of fetal fibrinogen production.

Fetal proteins may be re-expressed postnatally in human tissues, not only in association with malignancy (Fishman, 1976) but also with simple regenera-

tion (Jerry *et al.*, 1976). Thus a marked rise in fetal haemoglobin is not only typical of juvenile chronic myeloid leukaemia (Maurer *et al.*, 1972) but has also been described in association with the early phase of successful bone marrow transplantation (Alter *et al.*, 1976). Indeed, in the case of alpha-fetoprotein, serum levels may be as high in neonatal hepatitis as are ever recorded in PLC (Kang *et al.*, 1972; Zeltzer *et al.*, 1974).

On the basis of these interrelationships, it can be postulated that DF in liver disease results from derepression, in hepatocytes, of fetal genes coding for fibrinogen production; and, further, that this occurs only in proliferating liver tissue, *whether malignant or benign*, although it may require that the constituent cells have a short mean generation time and high growth fraction. In this regard it is of considerable interest that, in an experimental hepatoma model, the serum alpha-fetoprotein concentration was found to relate directly to the growth rate of the tumour (Sell *et al.*, 1974), and it has been proposed that the detection of raised levels of serum alpha-fetoprotein, in human non-neoplastic hepatic disease, may result from liver cell regeneration (Zeltzer *et al.*, 1974; Bloomer *et al.*, 1975).

Thus may be explained our observation that DF is not a feature of clinically overt PLC in Scotland but is associated with the tumour in Africans, in whom the disease is more rapidly progressive (Purves, 1976). Furthermore, in benign intrinsic liver disease, DF regresses with clinical resolution, as has been our experience and that of others (Soria *et al.*, 1970; Aiach *et al.*, 1973; Green *et al.*, 1976). By contrast, DF is not a feature of 'secondary' liver disease, for example metastatic carcinoma. In this circumstance, employing accepted criteria (Popper, 1954), convincing histological evidence of liver cell regeneration, adjacent to the metastases, was not detected in a survey of postmortem material from 15 patients who had secondary carcinoma in the liver. Indeed, the dominant features in those hepatocytes adjacent to the expansile tumour deposit appeared to be degenerative in nature.

Prolongation of the RT is more difficult to explain. We suggest that this represents a separate though possibly related abnormality, and we postulate that it reflects the release, from dead and dying hepatocytes, of material which does not exhibit fibrin-fibrinogen related antigen but which inhibits the action of reptilase on fibrinogen. Support for this hypothesis is provided by the finding, in the present study, that the concentration of serum aspartate aminotransferase was significantly higher in chronic liver disease patients with prolonged RT than in patients in whom the RT was normal ($P \ll 0.01$,

Wilcoxon's one-tailed test). By contrast there was no demonstrable association between DF and serum aspartate aminotransferase concentration.

Thus a prolonged RT occurs in the majority of patients with chronic parenchymal liver disease (Green *et al.*, 1976), in many patients with secondary hepatic damage who do not have DF (Green *et al.*, 1976), and in virtually all patients with PLC irrespective of the presence of DF. Again, resolution of benign liver disease has been accompanied by restoration of the RT to normal (Soria *et al.*, 1970; Green *et al.*, 1976) in accordance with our own findings.

Although neither DF nor a prolonged RT is indicative of PLC, a pattern of progressive abnormality on serial sampling may point to this diagnosis. We have studied one such patient in whom a histological diagnosis of micronodular cirrhosis was made in 1975. In June 1976 he presented with a considerable increment in hepatomegaly. ^{99m}Tc sulphur colloid and ⁷⁵Se selenomethionine scanning and repeated ultrasonography suggested the presence of PLC, and a probable tumour circulation was demonstrable by hepatic arteriography. Unfortunately this could not be confirmed by percutaneous and laparoscopic biopsies. Results of repeated estimations on this patient are shown in the Figure.

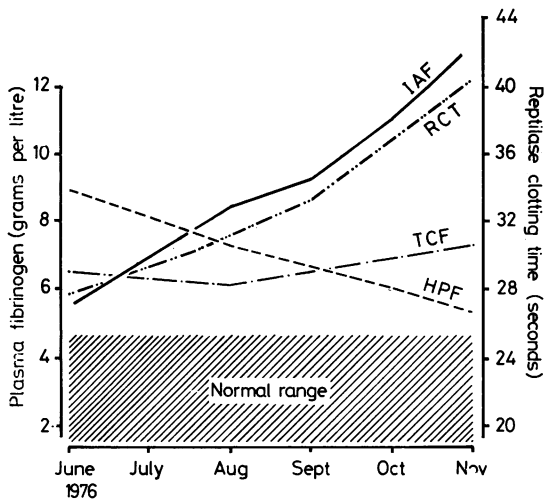


Figure Results of investigations in one patient, June-November 1976: RCT = reptilase clotting time; IAF = immunoassayable fibrinogen; TCF = thrombin clottable fibrinogen; HPF = heat-precipitable fibrinogen.

Alpha-fetoprotein has never been demonstrable in his serum by immunodiffusion nor by immunoelectrophoresis. Indeed, no patient in the present study had a serum alpha-fetoprotein level detectable

by the former technique, which has a sensitivity of 3000 ng per ml (Bloomer *et al.*, 1975).

We suggest that all patients with chronic parenchymal liver disease, but particularly males with haemochromatosis and alcohol-induced organ damage, be subjected to routine serial screening for DF and prolonged RT. In the event that progressive abnormalities are identified, every effort should be made to define and localise the PLC with a view to early and curative surgical removal of the tumour in suitable patients.

Our ongoing studies are directed to this end and to the further evaluation of these techniques, in patients with acute hepatitis, as potential predictors of clinical disease evolution.

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References

- Aiach, M., Rogé, J., Busy, M. F., Durand, H., Guéroult, N., Chanrion, C., Leclerc, M., and Justin-Besançon, L. (1973). Dysfibrinogénémies acquises et affections hépatiques. *Semaine des Hôpitaux de Paris*, **49**, 183-197.
- Alter, B. P., Rapoport, J. M., Huisman, T. H. J., Schroeder, W. A., and Nathan, D. G. (1976). Fetal erythrocytosis following bone marrow transplantation. *Blood*, **48**, 843-853.
- Barr, R. D., Ouna, N., Simpson, J. G., and Bagshawe, A. F. (1976). Dysfibrinogenaemia and primary hepatocellular carcinoma. *Quarterly Journal of Medicine*, **45**, 647-659.
- Bloomer, J. R., Waldmann, T. A., McIntire, K. R., and Klatskin, G. (1975). α -Fetoprotein in non-neoplastic hepatic disorders. *Journal of the American Medical Association*, **233**, 38-41.
- Fahey, J. L., and McKelvey, E. M. (1965). Quantitative determination of serum immunoglobulins in antibody-agar plates. *Journal of Immunology*, **94**, 84-90.
- Fishman, W. H. (1976). Activation of developmental genes in neoplastic transformation. *Cancer Research*, **36**, 3423-3428.
- Gralnick, H. R., Abrams, E., and Krolikowski, F. J. (1975). Acquired dysfibrinogenemia in hepatocellular carcinoma (HC) (Abstract). *Clinical Research*, **33**, 338 A.
- Gralnick, H. R., and Finlayson, J. S. (1972). Congenital dysfibrinogenemias. *Annals of Internal Medicine*, **77**, 471-473.
- Gralnick, H. R., Givelber, H. M., Shainoff, J. R., and Finlayson, J. S. (1971). Fibrinogen Bethesda: a congenital dysfibrinogenemia with delayed fibrino-

- peptide release. *Journal of Clinical Investigation*, **50**, 1819-1830.
- Green, G., Thomson, J. M., Dymock, I. W., and Poller, L. (1976). Abnormal fibrin polymerization in liver disease. *British Journal of Haematology*, **34**, 427-439.
- Jerry, L. M., Lewis, M. G., Rowden, G., Sullivan, A. K., Pitzele, R., and Law, T. (1976). Fetal antigens in non-neoplastic conditions. *Cancer Research*, **36**, 3446-3452.
- Kang, K. Y., Higashino, K., Takahashi, Y., Hashinotsume, M., and Yamamura, Y. (1972). α -Fetoprotein in infantile diseases. *Clinica Chimica Acta*, **42**, 175-180.
- Lane, D. A., Scully, M. F., Thomas, D. P., Kakkar, V. V., Wolf, I. L., and Williams, R. (1977). Acquired dysfibrinogenemia in acute and chronic liver disease. *British Journal of Haematology*, **35**, 301-309.
- Maurer, H. S., Vida, L. N., and Honig, G. R. (1972). Similarities of the erythrocytes in juvenile chronic myelogenous leukaemia to fetal erythrocytes. *Blood*, **39**, 778-784.
- Millar, H. R., Simpson, J. G., and Stalker, A. L. (1971). An evaluation of the heat precipitation method for plasma fibrinogen estimation. *Journal of Clinical Pathology*, **24**, 827-830.
- Ogston, C. M., and Ogston, D. (1966). Plasma fibrinogen and plasminogen levels in health and in ischaemic heart disease. *Journal of Clinical Pathology*, **19**, 352-356.
- Popper, H. (1954). Liver disease—morphologic considerations. *American Journal of Medicine*, **16**, 98-117.
- Purves, L. R. (1976). Alpha-fetoprotein and the diagnosis of liver cell cancer. In *Liver Cell Cancer*, edited by H. M. Cameron, C. A. Linsell, and G. P. Warwick, pp. 61-79. Elsevier, Amsterdam.
- Sell, S., Wepsic, H. T., Nickel, R., and Nichols, M. (1974). Rat alpha₁-fetoprotein. IV. Effect of growth and surgical removal of Morris hepatoma 7777 on the serum α_1 -F concentration of Buffalo rats. *Journal of the National Cancer Institute*, **52**, 133-137.
- Soria, J., Soria, C., Samama, M., Coupier, J., Girard, M. L., Bousser, J., and Bilski-Pasquier, G. (1970). Dysfibrinogénémies acquises dans les atteintes hépatiques sévères. *Coagulation*, **3**, 37-44.
- Straub, P. W. (1963). A study of fibrinogen production by human liver slices *in vitro* by an immunoprecipitin method. *Journal of Clinical Investigation*, **42**, 130-136.
- Verhaeghe, R., Van Damme, B., Molla, A., and Vermynen, J. (1972). Dysfibrinogenemia associated with primary hepatoma. *Scandinavian Journal of Haematology*, **9**, 451-458.
- Zeltzer, P. M., Neerhout, R. C., Fonkalsrud, E. W., and Stiehm, E. R. (1974). Differentiation between neonatal hepatitis and biliary atresia by measuring serum-alpha-fetoprotein. *Lancet*, **1**, 373-375.