Some chemical indices of diabetic vascular disease

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Summary

Some chemical indices which could be related to diabetic complications have been assessed in 105 diabetics. In juvenile (insulin-dependent) diabetics with retinopathy, cardiovascular disease or nephropathy, levels of fibrinogen, fibrin monomer, beta-lipoprotein, cholesterol and alpha-2-macroglobulin were raised. Only in part is the explanation due to a rise of fibrinogen and cholesterol with age. Among twenty-six patients with a plasma fibrinogen over 400 mg/100 ml, eleven sustained a major cardiovascular catastrophe within the next 2 years. Alpha-1-acid-glycoprotein was raised in these eleven and beta-2-glycoprotein generally in juveniles with cardiovascular disease. Triglycerides were elevated in all groups, but notably in patients with Grade I retinopathy with venous dilation. There was a suggestion that triglycerides were related inversely to dietary carbohydrate in mature onset diabetics, and serum cholesterol in juveniles was related to insulin dosage. The part of lipids in vascular disease is then discussed. An interesting positive correlation was found between fibrin monomer, itself an indication of in vivo thrombin formation, and beta-lipoprotein.

Although alpha-2-macroglobulin was elevated in juveniles, especially with complications, this did not apply to maturity onset cases and therefore no direct relation to diabetic vascular disease can be suggested. Moreover, alpha-2-macroglobulin levels were normal in acromegaly.

Many investigators have sought biochemical estimations which will correlate with both the status and progression of diabetic vascular disease, since apart from visualization of retinopathy, clinical assessment relies as much upon intuition as upon objective analysis. The situation is complicated by the associations of atherosclerosis and hypertensive arteriosclerosis with the specific microangiopathy, and the tendency to early myocardial infarction and peripheral cerebrovascular occlusive episodes. We have made our own re-assessment of some biochemical values, choosing for the purpose estimations that are simple, since such tests should have wider application in routine work. The report presents the results of a survey of 105 diabetic patients attending the out-patient clinic on whom estimations were made of serum lipids, fibrinogen and fibrin monomer and of serum alpha-2-macroglobulin and of glycoproteins.

Diabetic microangiopathy is the result of endothelial cell proliferation in capillaries, arteries and venules, and there are increased amounts of glycoprotein in the vessel walls (Ditzel, 1968) and in the thickened and excessively permeable basement membrane (Garner, 1970). The basement membrane changes rest in turn on a metabolic abnormality of smooth muscle mural cells, and in the renal glomerulus on the epithelial cells (Walker, 1968) but whether this is due to insulin lack or excess (Jackson, Van Mieghem & Van Keller, 1972), a relative excess of growth hormone (Lundback et al., 1970; Beaumont et al., 1971) or an associated genetic defect (Siperstein, 1970) is not known.

Retinopathy probably depends primarily on arteriolar occlusion leading to localized areas of ischaemia (Heath, 1970) and hence to capillary shunts (Cogan & Kuwabara, 1963). These occlusions are possibly due to lipid thrombi (Chester & Banker, 1967) but high viscosity may be a factor (Chazan, 1963). Lipid and polysaccharide (Ditzel, 1967) accumulation certainly plays a part in exudative and nodular renal lesions (Salinas-Madrigal, Pirani & Pollak, 1970; Ireland, 1968). Lipids are also recognized as predictors of atherosclerotic disease (Stamler, 1967) since the consensus of opinion is that atheromatous plaques are due to the infiltration of arterial walls by lipoproteins (Getz, Vesselinovitch & Wissler, 1969; Brown, 1969). There can also be lipid synthesis within the wall (Chernick, Srere & Chaikoff, 1949; Stout, 1970). Fibrinogen levels are increased secondarily as a result of atherosclerotic vascular disease (Pilgeram, Schram & Mills, 1960;
Hampton et al., 1966) but possibly also in diabetics as a result of their microangiopathic process.

Methods

Blood samples were taken from 105 diabetic patients attending the 5.00 p.m. evening outpatient clinic. For the majority of patients this was 4 hr after the last main meal. They were classified clinically as juvenile insulin-dependent or maturity onset diabetics and clinical assessment was made as to the grade of retinopathy present and the presence or absence of cardiovascular disease or nephropathy. Grade I retinopathy signifies the presence of outstanding venous dilatation, Grade II the finding of numerous microaneurysms, Grade III the presence of microaneurysms with hard exudates and Grade IV fibroproliferative changes and neovascularization. Those listed as having cardiovascular disease were patients with a history of angina or of myocardial infarction, with hypertension or with a story of peripheral or cerebrovascular occlusions. Nephropathy was presumed by the finding of persistent proteinuria (greater than 0.1 g/day in the absence of urine infection) and in the majority of these patients renal biopsy had been performed.

Plasma samples were analysed for fibrinogen content (Ratnoff & Menzie, 1951) and for fibrin monomer complexes as optical density units (Lipinski & Worowski, 1968). Fibrin monomer is the first breakdown product of fibrinogen, with the A and B peptides removed, and is a determinant of the erythrocyte sedimentation rate (Lipinski et al., 1969). Serum samples were estimated for the content of beta-lipoprotein (Dangerfield & Faulkner, 1964), for cholesterol (Henly, 1957), for triglycerides (Van Handel & Zilversmit, 1957) and for estimation by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) of alpha-2-macroglobulin, alpha-1-glycoprotein and beta-2-glycoprotein. Fibrin monomer and plasma fibrinogen have also been estimated in a group of normal people and the higher values are derived from postoperative blood samples. As an addendum to the study alpha-2-macroglobulin levels were also estimated in the sera of seventeen acromegal patients in comparison with thirteen control sera by analysis of alpha-2-macroglobulin via its antitryptic activity (Ganrot, 1966).

Results and comment

Of the 105 diabetics fifty had no retinopathy, fourteen had marked venous dilatation, thirteen micro-anerysms, twenty hard exudates and eight proliferative retinopathy. Twenty-one patients had cardiovascular complications and twenty-two juveniles and ten maturity-onset diabetics had nephropathy. There were in all ten patients with hypertension and eleven patients who, in a 2 year period after the initial investigations, either died or had a serious cardiovascular accident. Table 1 gives the clinical subdivisions and results of the various estimations in comparison with the type of complication. The mean age and weight in pounds of the sub-groups are also noted. Particularly outstanding are the significantly higher levels of

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<tr>
<th>Table 1. Grade of vascular disease</th>
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<td>Normal</td>
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<tr>
<td>Fibrinogen (mg/100 ml)</td>
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<td>300 ± 73</td>
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<td>356 ± 105</td>
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<td>Fibrin monomer (optical density units)</td>
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<td>β-lipoprotein (mg/100 ml)</td>
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<td>365 ± 85</td>
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<td>380 ± 190</td>
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<td>Cholesterol (mg/100 ml)</td>
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<td>234 ± 47</td>
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<td>Triglyceride (mg/100 ml)</td>
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<td>199 ± 97</td>
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<td>α-2-macroglobulin (mg/100 ml)</td>
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<td>240 ± 91</td>
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<td>α-1-acid glycoprotein (mg/100 ml)</td>
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<td>95 ± 36</td>
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<td>β-2-glycoprotein (mg/100 ml)</td>
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Significance values by Student’s 't' test.
fibrinogen and fibrin monomer, beta-lipoprotein and cholesterol and the higher levels of alpha-2-macroglobulin in juvenile diabetics with complications. Beta-2-glycoprotein was elevated in juveniles with cardiovascular disease. Triglyceride levels were high in all the groups but did not achieve significant group differences.

Table 2 gives the results of the same estimations in relation to the type of retinopathy. High levels of beta-lipoprotein and of cholesterol, fibrinogen and alpha-2-macroglobulin were found in patients with Grade III and IV retinopathy. Of interest is the finding of higher triglyceride levels in patients whose retinas showed marked venous dilation. These Grade I patients also showed elevated plasma fibrinogen and fibrin monomer complexes.

Table 3 lists the results of the same investigations for the ten patients with hypertension, who clearly did not differ greatly from the patients as a whole, although their triglyceride levels were a little high, and for the eleven patients who in the 2 years of follow-up sustained a catastrophe. These latter are distinguished again by high serum triglycerides but more particularly by a high fibrinogen and an α-1-acid glycoprotein.

Table 4 gives regression equations which define the rise of plasma fibrinogen with age in the diabetics and also among normal patients: the rate of rise of plasma fibrinogen with age is greater among the diabetics. Regression equations are also given for the rise of cholesterol with age which is very steep and significant in the juvenile group. Only slow rises of triglyceride with age are apparent but this no doubt is due to the use of postprandial sampling. What is clear is that the values in Table 1 that attain the greatest significance, namely those for the plasma fibrinogen and cholesterol, are precisely those that show the greatest increase with age of the patient, and with duration of diabetes. However, the figures given in parentheses in Table 1 for the
fibrinogen and cholesterol values are the mean values expected on the basis of age, these values being derived from the regression equations. In spite of such correction the significant values in Table 1 still exceed expectation and the inference must be that the vascular pathology or its cause is also exerting an effect.

Table 5 is an analysis of how far serum cholesterol and postprandial triglycerides could be related to body weight. In fact regression lines of these parameters were not significant, but by setting arbitrary divisions of above or below 140 lb weight, in the case of juvenile diabetics there was then a highly significant association with cholesterol of over 300 mg/100 ml and postprandial triglyceride of over 200 mg/100 ml.

In addition, cholesterol in juvenile diabetics could be related in part to their total insulin dosage, as is shown in Fig. 1. Postprandial triglyceride showed no such relation but in mature onset diabetics was found to be related inversely to the dietary carbohydrate intake in grams (Fig. 2). Hence, contrary to expectation, mature onset diabetics on the strictest diets had the highest postprandial serum triglyceride levels. The only possible explanation is that the effect is the result of mobilization of fatty acids in starvation.

In our normal patients there was a good correlation between the level of fibrin monomer and the plasma fibrinogen \( (r = +0.52, P < 0.001) \), as is shown in Fig. 3. However, surprisingly, for diabetics the correlation was less certain \( (r = +0.27, P < 0.001) \) (Fig. 4) but in them the level of fibrin monomer correlated better with their level of beta-lipoprotein \( (r = +0.52, P < 0.001) \) (Fig. 5). In turn for all patients beta-lipoprotein correlated well with the level of triglyceride \( (r = +0.72, P < 0.001) \), and less well with the serum cholesterol \( (r = +0.6, P < 0.001) \). Analysis of partial regression equations gave a coefficient of partial correlation of fibrin monomer against beta-lipoprotein when the plasma fibrinogen is kept constant of \( r = +0.4 \) and the coefficient of multiple correlation of fibrin monomer against beta-lipoprotein and plasma fibrinogen was \( R = +0.36 \). No relation could be established between serum cholesterol or serum triglyceride and plasma fibrinogen (cf. Hart, Thorp & Cohen, 1971).

Further studies on the alpha-2-macroglobulin levels showed that the level was elevated even in young juvenile diabetics and that there was no relationship to age or duration of diabetes. Even higher levels were observed in juvenile patients with retinopathy or with nephropathy as assessed by proteinuria but this did not apply to maturity-onset cases. Normal levels were 235 mg/100 ml \((±87.0)\) and in acromegalic patients the alpha-2-macro-
Chemical indices of diabetic vascular disease

**Fig. 2.** Serum triglyceride in mature diabetics related to carbohydrate intake.
\[ y = 0.88x + 318; r = -0.3; s = 85.8; P > 0.025. \]

**Fig. 3.** Normal relation of fibrin monomer to fibrinogen.
\[ y = 0.14x + 20.6; r = +0.78; P < 0.001. \]

**Fig. 4.** Juvenile diabetics. Relation of fibrin monomer to fibrinogen.
\[ y = 0.046x + 43.7; r = +0.27; P < 0.001. \]

**Fig. 5.** Relation between fibrin monomer and beta-lipoprotein for juvenile diabetics.
\[ y = 0.082x + 28.6; r = +0.52; P < 0.001. \]

globulin levels were 199±86.0 mg/100 ml. Levels in acromegals were therefore normal.

**Discussion**

It is apparent that the results of four simple tests taken in conjunction, namely plasma fibrinogen, fibrin monomer, beta-lipoprotein and cholesterol, are related to complicated disease in the juvenile diabetics. Only in part do the findings seem to be due to the accelerated rise of fibrinogen and cholesterol with age. Nevertheless, it is undecided whether
the biochemical values reflect cause or effect of vascular disease. What is clear is that they can be used as predictors. Thus, in the eleven patients who suffered a catastrophe the fibrinogen was high before the event.

Although the use of postabsorptive plasma would be expected to limit the value of the lipid estimations, we deliberately chose to use such samples, as obtaining overnight fasting samples in a diabetic population raises organizational problems. Moreover, it is the level of the lipids in the postabsorptive state and not the fasting level, that probably determines the cardiovascular complications. Furthermore, it has been shown that postprandial serum triglycerides do correlate well with fasting levels (Zeveers, Yaron, & Groen, 1968). These authors state that a postprandial triglyceride of over 224 mg/100 ml is abnormal. It will be seen therefore that many of the diabetics have serum triglycerides that are close to the upper limit of normality. This is not surprising as it is now known that some 22% of diabetics have Type II hyperlipoproteinaemia, 63% Type IV, 3% Type V and only 22% have normal lipoproteins (Zorilla, Hernandez & Serrano, 1970; Wilson et al., 1970). Lipoprotein typing is based on the classification of Fredricksen, Levy & Lees (1967) but must be performed on fasting serum samples.

Previous studies of lipids in diabetics show the association of elevated lipids with atherosclerosis but the relationship to microangiopathy is less clear (Albrink, Lavietes & Man, 1963; Lowy & Barach, 1958; Introzzi, Bernascani & Buscarani, 1958; Bergqvist, 1970). Although the results are applicable to population groups, data do not segregate individuals. In a variety of conditions elevated beta-lipoprotein is associated with an increase of fibrinogen and increased heparin tolerance (Mittel'shtedt et al., 1963). Both cholesterol and triglyceride levels relate to the incidence of coronary artery disease (Brown, Kinch & Doyle, 1965) and have predictive value in the population (Stamler, 1967), although the relative value of each may be disputed (Mills, Taylour & Wilkinson, 1969). This is logical since lipoproteins are both transported through the arterial wall from the plasma and synthesized in situ (Holland, 1967).

Elevated triglycerides are stated to occur in diabetics who are obese, have atherosclerosis, or who receive a very high carbohydrate or fat diet, but generally subside when diabetes is under control. On the contrary our results show that in mature diabetics the postprandial triglycerides are highest in those patients on the strictest diets. This may be a reflection of the afore mentioned factor of obesity, as might also be inferred from Table 5, but the suggestion cannot be ignored that fatty acid mobilization as a result of starvation is leading to increased triglyceride synthesis, which in turn might aggravate vascular disease.

The suggestion from Fig. 1 that the serum cholesterol level is in part dependent upon the insulin dosage is also disquieting. This might explain why the cholesterol is rising so rapidly with age in the juvenile diabetics, and in turn the inevitability of atherosclerotic vascular disease. The additional point is also to be noted that the cholesterol is higher when the body weight exceeds 140 lb, as is the case for triglyceride. Of course insulin has also been incriminated in triglyceride synthesis and liability to atherosclerosis (Stout & Vallance-Owen, 1969).

That lipids can be elevated merely as a result of diabetic nephropathy (Engelberg, Gofman & Jones, 1952) as in any type of nephrosis, is accepted but the relation of lipids to retinopathy is not so certain, although lipid thrombi would explain the arteriolar occlusions (Chester & Banker, 1967; Innaccone & Kornerup, 1954; Pope, 1960). Keiding et al. (1952) were able to show that serum levels of cholesterol or of δ2 12-20 lipoproteins are characteristically associated with the degree of retinopathy. Our results certainly confirm that lipoprotein levels are correlated with the grade of retinopathy and our finding that the triglyceride levels are higher in those diabetics with dilated retinal veins is of interest, since a raised chylomicron count and triglyceride is known to cause intravascular red cell aggregation and vascular stasis (Chazan, 1963) accompanied by an increase of blood viscosity (Skovborg et al., 1966). Moreover fibrin monomer was also high in this group and fibrin monomer is known to affect red cell aggregation (Lipinski et al., 1969).

Ditzel (1967) has stressed the relation of erythrocyte aggregation to diabetic microangiopathy. Elevated viscosity is however dependent on increase of fibrinogen and alpha and beta globulins (Skovborg et al., 1966). Alpha-2-macroglobulin has also been reported to be high in some diabetics (James, Jonsson & Fudenberg, 1966) and it has long been known that alpha-2-globulins are raised in complicated disease (Ejarque, Marble & Tuller, 1959; Skanse, 1963). Our results show that higher levels of alpha-2-macroglobulin occur in juvenile patients with advanced retinopathy, nephropathy and cardiovascular complications and accord with the results of Cleve et al. (1968). Since nephropathy is an almost inevitable accompaniment of diabetic vascular disease the raised alpha-2-macroglobulin might be a reflection of proteinuria as is the case in nephrotic syndrome (Kluthe, Hagemann & Kleine, 1967) or of the vascular permeability which is a feature of diabetic vascular disease (Trap-Jensen, 1970). However, since alpha-2-macroglobulin levels cannot be made to correlate with proteinuria alone and are
hardly elevated in mature diabetics the finding is probably a result of the altered metabolism of diabetes and not related to the pathogenesis of angiopathy. Recently it has been found (Muller, 1970) that both the fractional and absolute catabolic rate and hence also the synthesis rate of alpha-2-macroglobulins is increased in diabetics.

The hope that study of alpha-2-macroglobulin levels might establish how early vascular disease appears has been abrogated by the finding that levels can be high at the time of diagnosis, and in adolescent diabetics the level may merge with the elevation that is normally found in childhood (Ganrot & Schersten, 1967). This raised the possibility that growth hormone might be a determinant of alpha-2-macroglobulin synthesis, particularly as alpha-2-macroglobulin is a growth-hormone-binding protein (Hadden & Proust, 1964), and abnormally high growth hormone responses are known to occur in juvenile diabetics (Yde, 1969). However, our finding that alpha-2-macroglobulin levels are normal in patients with acromegaly refutes this theory. It is moreover salutary to recall that diabetic vascular disease is not florid in acromegaly.

Alpha-1-acid glycoprotein is known as an acute reactant protein but the function of beta-2-glycoprotein is unknown. It is interesting that beta-2-glycoprotein levels were elevated in juveniles with cardiovascular disease. The reason for studying these glycoproteins was that protein-bound carbohydrate has been shown to be elevated in both the serum and vessel walls in atherosclerosis (Muller-Spreer, Werber & Voigt, 1960; Schonebeck, Werber & Voigt, 1962).

Hypercoagulability in diabetics as shown by an increase of fibrinogen and factors V, VII, VIII together with heparin resistance according to the grade of diabetic complications has been previously noted by Valdorp-Hansen (1967) but fibrinolysis in diabetics has been reported to be normal (McKay & Humé, 1964; Cash & McGill, 1964). This may not however be true when subdivisions of clinical diabetes are considered. The results of Table 3 suggest that fibrinogen synthesis rates are probably increased in diabetics as a result of vascular disease, although this remains to be proven. A higher fibrinogen together with elevation of other coagulation factors will explain the liability to thrombotic episodes, especially as platelet adhesiveness is also increased in diabetics (Pegrum, Wolff & Ashton, 1967). Fibrin monomer complexes have been found to be elevated in our young diabetics and the results suggest that these are related to elevation of beta-lipoprotein levels, possibly because fatty acids and lipids promote platelet aggregation (Hoak, Warner & Connor, 1967). Indeed it has recently been reported that platelet factor IV release from platelets is greater in diabetics (Chmielewski & Farbiszewski, 1970) no doubt because coating of platelets by beta-lipoprotein enhances their sensitivity to aggregating agents.

In conclusion therefore it appears that elevation of lipids is closely correlated with and may therefore explain some features of diabetic vascular disease. Thus elevated triglycerides are associated with retinal venous dilation. Elevation of lipids secondary to nephropathy can be expected to establish a vicious cycle in the course of the disease. Finally the higher levels of lipids can explain the increased platelet adhesiveness and liability to aggregation, and the increased production of fibrin monomer complexes as a result of this may be the reason why fibrinogen synthesis is increased.

Acknowledgments

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References


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