Some chemical indices of diabetic vascular disease

E. N. WARDLE
M.A., M.B., M.R.C.P.

D. A. PIERCY

J. ANDERSON
M.B., M.R.C.P.

Department of Medicine, Royal Victoria Infirmary, Newcastle-upon-Tyne NE1 4LP

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 and 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 Miegheem & 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 out-patient 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 dilation, Grade II the finding of numerous microaneurysms, Grade III the presence of microaneurysms with hard exudates and Grade IV fibroproliferative changes and neo-vascularization. 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 acromegalic 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 dilation, thirteen micro-aneurysms, 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

TABLE 1. Grade of vascular disease

	Normal		Retinopathy		Cardiovascular		Nephropathy	
	Juvenile	Mature	Juvenile	Mature	Juvenile	Mature	Juvenile	Mature
Fibrinogen (mg/100 ml)	300±73 (320)	356±105 (320)	410±104 (340)	408±80 (355)	458±72 (360)	396±133 (355)	478±134 (360)	440±102 (355)
					$P < 0.001$		$P < 0.05$	$P < 0.05$
Fibrin monomer (optical density units)	0.60±0.10	0.55±0.17	0.65±0.19	0.62±0.21	0.78±0.20	0.70±0.19	0.73±0.18	0.78±0.16
					$P < 0.005$		$P < 0.001$	$P < 0.005$
β lipoprotein (mg/100 ml)	365±85	380±190	415±205	422±95	555±200	410±110	645±250	372±170
					$P = 0.001$		$P < 0.001$	
Cholesterol (mg/100 ml)	234±47 (265)	227±61 (240)	308±111 (295)	265±75 (248)	385±135 (335)	271±86 (248)	389±135 (335)	260±108 (248)
			$P < 0.005$		$P < 0.001$		$P < 0.001$	
Triglyceride (mg/100 ml)	199±97	220±95	205±76	240±116	214±52	236±102	268±157	236±100
α-2-macroglobulin (mg/100 ml)	240±91	242±96	316±105 $P < 0.05$	265±73	336±98 $P < 0.05$	208±87	345±84 $P < 0.01$	244±114
α-1-acid glycoprotein (mg/100 ml)	95±36	100±32	100±39	110±30	107±33	126±54	118±42	175±60
β-2-glycoprotein (mg/100 ml)	25±12	28±12	34±14	27±12	40±16 $P < 0.05$	22±8	35±12	31±16
Age	34±13	59±12	40±13	65±11	48±12	66±13	47±7	63±13
Weight (lb)	132±22	150±26	143±23	140±21	144±18	139±26	144±19	142±29
Total numbers	26	24	30	25	7	14	22	10

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 retinae 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

TABLE 2. Grade of retinopathy

Retinopathy grade	0	1	2	3	4
Fibrinogen (mg/100 ml)	277 $\pm$ 73	390 $\pm$ 145 <i>P</i> < 0.005	348 $\pm$ 119 <i>P</i> < 0.02	418 $\pm$ 98 <i>P</i> < 0.001	351 $\pm$ 60 <i>P</i> < 0.02
Fibrin monomer (optical density units)	0.55 $\pm$ 0.16	0.7 $\pm$ 0.24 <i>P</i> < 0.02	0.65 $\pm$ 0.12 <i>P</i> = 0.05	0.61 $\pm$ 0.23	0.62 $\pm$ 0.14
β -lipoprotein (mg/100 ml)	345 $\pm$ 180	412 $\pm$ 210 <i>P</i> = 0.05	392 $\pm$ 190	440 $\pm$ 270 <i>P</i> < 0.001	540 $\pm$ 180 <i>P</i> < 0.001
Cholesterol (mg/100 ml)	228 $\pm$ 52	270 $\pm$ 42 <i>P</i> < 0.02	238 $\pm$ 65	276 $\pm$ 62 <i>P</i> < 0.005	356 $\pm$ 56 <i>P</i> < 0.001
Triglyceride (mg/100 ml)	199 $\pm$ 106	262 $\pm$ 101 <i>P</i> < 0.05	250 $\pm$ 156	249 $\pm$ 126	229 $\pm$ 102
α -2-macroglobulin (mg/100 ml)	224 $\pm$ 84	240 $\pm$ 121	240 $\pm$ 78	286 $\pm$ 103 <i>P</i> < 0.05	370 $\pm$ 84 <i>P</i> = 0.05
α -1-acid glycoprotein (mg/100 ml)	97 $\pm$ 52	100 $\pm$ 48	124 $\pm$ 53	101 $\pm$ 37	130 $\pm$ 41
β -2-glycoprotein (mg/100 ml)	25 $\pm$ 11	27 $\pm$ 12	20 $\pm$ 8	33 $\pm$ 16	32 $\pm$ 16
No. of patients	50	14	13	20	8*

* Half of the patients in this group were already receiving Atromid-S. Significance analysed by Student's 't' test.

TABLE 3. Comparison of results in hypertensive patients with those sustaining a new cardiovascular episode

	Hypertension (10)	Deaths or new cardiovascular accident in 2 year follow-up (11)	
Fibrinogen	345 $\pm$ 68	443 $\pm$ 118	<i>P</i> < 0.05
Fibrin monomer	0.66 $\pm$ 0.07	0.69 $\pm$ 0.17	
β -lipoprotein	323 $\pm$ 123	311 $\pm$ 130	
Cholesterol	307 $\pm$ 88	266 $\pm$ 130	
Triglyceride	232 $\pm$ 95	242 $\pm$ 165	
α -2-macroglobulin	244 $\pm$ 34	204 $\pm$ 51	
α -1-acid glycoprotein	108 $\pm$ 20	124 $\pm$ 17	<i>P</i> < 0.1
β -2-glycoprotein	25 $\pm$ 8	27 $\pm$ 15	
Age	54 $\pm$ 8	64 $\pm$ 13	
Weight	138 $\pm$ 21	142 $\pm$ 22	

TABLE 4. Age-related changes

Fibrinogen			
Normal	$x = 2.2x + 200$	$r = +0.45$	$P < 0.001$
Juvenile	$x = 2.8x + 227$	$r = +0.405$	$0.001 < P < 0.005$
Mature	$x = 3.2x + 148$	$r = +0.44$	$P < 0.001$
Cholesterol			
Juvenile	$y = 5x + 98$	$r = +0.98$	$P < 0.001$
Mature	$y = 0.9x + 187$	$r = +0.22$	$P < 0.05$
Postprandial triglyceride			
Juvenile	$x = 1.21x + 177$	$r = +0.05$	—
Mature	$x = 1.7x + 117$	$r = +0.23$	$P < 0.1$

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

TABLE 5. Relation of lipids to body weight over 140 lb

	Juvenile	Mature
Cholesterol		
> 300 mg/100 ml	$\chi^2 = 10.6$ $P < 0.005$	$\chi^2 = 0.38$ NS
Triglyceride		
> 200 mg/100 ml	$\chi^2 = 9.72$ $P < 0.005$	$\chi^2 = 3.82$ $P = 0.05$

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

FIG. 1. Juvenile diabetics. Relation of serum cholesterol to insulin dosage.

$$y = 1.15x + 199; r = +0.3; s = 58; P < 0.02.$$

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-

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. 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 acromegalics 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

FIG. 3. Normal relation of fibrin monomer to fibrinogen.

$$y = 0.14x + 20.6; r = +0.78; P < 0.001.$$

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 (Zeevers, 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% diabetics have Type II hyperlipoproteinaemia, 63% Type IV, 3% Type V and only 22% have normal lipoproteins (Zorrilla, Hernandez & Serrano, 1970; Wilson *et al.*, 1970). Lipoprotein typing is based on the classification of Fredrickson, 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, Laviates & Man, 1963; Lowy & Barach, 1958; Introzzi, Bernasconi & 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 (Mittelshedt *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, T aylour & Wilkinson, 1969). This is logical since lipoproteins are both transported through the arterial wall from the plasma and synthesized *in situ* (Hollander, 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; Innacone & Kornerup, 1954; Pope, 1960). Keiding *et al.* (1952) were able to show that serum levels of cholesterol or of δ_f 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, Jonnson & 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 acromegals.

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 Valdorf-Hansen (1967) but fibrinolysis in diabetics has been reported to be normal (McKay & Hume, 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.

Acknowledgment

We are grateful to Professor R. Hall for access to sera from patients with acromegaly.

References

- ALBRINK, M.J., LAVIETES, P.H. & MAN, E.B. (1963) Vascular disease and serum lipids in diabetes mellitus. *Annals of Internal Medicine*, **58**, 305.
- BEAUMONT, P., HOLLOWES, F.C., SCHOFIELD, P.J., WILLIAMS, J.F. & STEINBECK, A.W. (1971) Growth hormone, sorbitol and diabetic capillary disease. *Lancet*, **i**, 579.
- BERGQUIST, N. (1970) Serum lipids in an ambulatory diabetic clientele. *Acta medica scandinavica*, **187**, 213.
- BERKMAN, J., RIFKIN, H. & ROSE, G. (1953) Serum polysaccharides in diabetes mellitus. *Journal of Clinical Investigation*, **32**, 415.
- BROWN, D.F., KINCH, S.H. & DOYLE, J.T. (1965) Serum triglycerides in health and in ischaemic heart disease. *New England Journal of Medicine*, **273**, 947.
- BROWN, D.F. (1969) Blood lipids and lipoproteins in atherogenesis. *American Journal of Medicine*, **46**, 691.
- CASH, J.D. & MCGILL, R.C. (1969) Fibrinolytic response to moderate exercise in young male diabetics and non-diabetics. *Journal of Clinical Pathology*, **22**, 32.
- CHAZAN, B.I. (1963) Intravascular red cell aggregation and the chylomicron count in diabetes. *Angiology*, **14**, 426.
- CHEARNICK, S., SRERE, P.A. & CHAIKOFF, I.L. (1949) The metabolism of arterial tissue. *Journal of Biological Chemistry*, **179**, 113.
- CHESTER, E.M. & BANKER, B.Q. (1967) The role of lipid thrombi in the pathogenesis of diabetic retinopathy. *Archives of Internal Medicine*, **120**, 397.
- CHMIELEWSKI, J. & FARBISZEWSKI, R. (1970) Platelet factor four release during human platelet aggregation in diabetic patients. *Thrombosis et diathesis haemorrhagica*, **24**, 203.
- CLEVE, H., ALEXANDER, K., MITZKAT, H.J., NISSEN, P. & SALZMANN, I. (1968) Serum glykoproteine beim Diabetes Mellitus. *Diabetologica*, **4**, 48.
- COGAN, D.G. & KUWABARA, T. (1963) Capillary shunts in the pathogenesis of diabetic retinopathy. *Diabetes*, **12**, 293.
- DANGERFIELD, W.G. & FAULKNER, G. (1964) Estimation of serum lipoproteins using sulphated polysaccharides. *Clinica chimica acta*, **10**, 123.
- DITZEL, J. (1967) Haemorrhological factors in the development of diabetic microangiopathy. *British Journal of Ophthalmology*, **51**, 793.
- DITZEL, J. (1968) Functional microangiopathy in diabetes mellitus. *Diabetes*, **17**, 388.

- EJARQUE, P., MARBLE, A. & TULLER, E.F. (1956) Proteins, lipoproteins and protein-bound carbohydrates in the serum of diabetic patients. *American Journal of Medicine*, **27**, 221.
- ENGELBERG, H., GOFMAN, J. & JONES, H. (1952) Serum lipids and lipoproteins in diabetic glomerulosclerosis. *Diabetes*, **1**, 425.
- FREDERICKSEN, D.S., LEVY, R.I. & LEES, R. (1967) Fat transport in lipoproteins. *New England Journal of Medicine*, **276**, 148, 215, 273, 281.
- GANROT, P.O. (1966) Estimation of α_2 -macroglobulin as trypsin-protein esterase. *Clinica chimica acta*, **14**, 493.
- GANROT, P.O., SCHERSTEN, B. (1967) Serum α_2 -macroglobulin concentration and its variation with age and sex. *Clinica chimica acta*, **15**, 113.
- GARNER, A. (1970) Pathology of diabetic retinopathy. *British Medical Bulletin*, **26**, 137.
- GETZ, G.S., VESSELINOVITCH, D., WISSLER, R.W. (1969) A dynamic pathology of atherosclerosis. *American Journal of Medicine*, **46**, 657.
- HAMPTON, J.W., MANTOOTH, J., BRANDT, E.N. & WOLF, S.G. (1966) Plasma fibrinogen patterns in patients with coronary atherosclerosis. *Circulation*, **34**, 1098.
- HADDEN, D.R. & PROUST, TH. E. (1964) A growth hormone binding protein in normal human serum. *Nature*, **202**, 1342.
- HART, A., THORP, J.M. & COHEN, H. (1971) Lipoprotein and fibrinogen studies in diabetes. *Postgraduate Medical Journal Suppl.* **48**, 1971, 435.
- HEATH, H. (1970) Experimentally induced retinopathies in relation to the problem of diabetes. *Analyst*, **82**, 286.
- HENLY, A.A. (1957) Estimation of serum cholesterol. *Analyst*, **82**, 286.
- HOAK, J.C., WARNER, E.D. & CONNOR, W.E. (1967) Platelets, fatty acids and thrombosis. *Circulation Research*, **20**, 11.
- HOLLANDER, W. & KRANSCH, D.M. (1967) The distribution of intravenously administered ^3H cholesterol in the arteries and other tissues. *Journal of Atherosclerosis Research*, **7**, 491.
- IANNACONE, A. & KORNERUP, T. (1954) Plasma lipids and diabetic retinopathy. *Acta medica scandinavica*, **138**, 411.
- INTROZZI, P., BERNASCANI, C. & BUSCARANI, L. (1958) Serum proteins, lipids and protein bound carbohydrates in vascular complications of diabetes mellitus. *Acta medica scandinavica*, **160**, 47.
- IRELAND, J.T. (1968) Kidney changes in diabetes. *Hospital Medicine*, **2**, 783.
- JACKSON, W.P.U., VAN MIEGHEM, W. & VAN KELLER, P. (1972) Insulin excess as the initial lesion in diabetes. *Lancet*, **i**, 1040.
- JAMES, K., JOHNSON, G. & FUDENBERG, H.H. (1966) The quantitative estimation of α_2 -macroglobulin in normal, pathological and cord sera. *Clinica chimica acta*, **14**, 207.
- KEIDING, N.R., MANN, G.V., ROOT, H.F., LAURY, E.Y. & MARBLE, A. (1952) Serum lipoproteins and cholesterol levels in normal subjects and in young patients with diabetes in relation to vascular complications. *Diabetes*, **1**, 434.
- KLUTHE, R., HAGEMANN, U. & KLEINE, N. (1967) The turnover of α_2 -macroglobulins in the nephrotic syndrome. *Vox Sanguinis*, **12**, 308.
- LIPINSKI, B. & WOROWSKI, K. (1968) Detection of soluble fibrin monomer complexes in blood by means of protamine sulphate test. *Thrombosis et diathesis haemorrhagica*, **20**, 44.
- LIPINSKI, B., WOROWSKI, K., MYSLIWIEC, M. & FARBISZEWSKI, R. (1969) Erythrocyte sedimentation and soluble fibrin monomer complexes. *Thrombosis et diathesis haemorrhagica*, **21**, 196.
- LOWY, A.D. & BARACH, J.H. (1958) Predictive value of lipoprotein and cholesterol determinations in diabetic patients who have developed cardiovascular complications. *Circulation*, **17**, 14.
- LUNDBAEK, K., CHRISTENSEN, N.J., JENSEN, V.A., JOHNSON, J., STEEN OLSEN, T., HANSEN, A.P., ORSKOV, H. & OSTERBY, R. (1970) Diabetes. Diabetic angiopathy and growth hormone. *Lancet*, **ii**, 131.
- MACKEY, N. & HUME, R. (1964) Fibrinolytic activity in diabetes mellitus. *Scottish Medical Journal*, **9**, 359.
- MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.
- MILLS, G.L., TAYLOUR, C.E. & WILKINSON, P.A. (1969) An analysis of the distribution of lipoprotein patterns in healthy men and in patients with myocardial infarction. *Clinica chimica acta*, **26**, 67.
- MITTELSTEDT, A.A., BAUMAN, L.K., KARPINSKAYA, V.M. & KNYAZEVA, G.R. (1963) Serum lipoproteins in patients with various circulatory disturbances. *Federal Proceedings*, **T240**.
- MULLER, H. (1970) Zum stoffwechsel des α_2 -Makroglobulin bei Diabetes Mellitus. *Klinische Wochenschrift*, **21**, 1276.
- MULLER-SPREER, H.C., WEBER, V. & VOIGT, K.D. (1960) Untersuchungen über die Höhe und Zusammensetzung der Eiweissgebundenen Kühlenhydrathaltigen Bestandteile in serum und Gefäßwand von Normal-personen und Arteriosklerotikern. *Klinische Wochenschrift*, **38**, 28.
- PEGURU, G.D., WOLFF, S. & ASHTON, W.L. (1967) Platelet adhesiveness in diabetes mellitus. *Journal of Clinical Pathology*, **20**, 845.
- PILGERAM, L.O., SCHRAM, A.C. & MILLS, D. (1960) Specificity of plasma fibrinogen changes for atherosclerosis. *Circulation*, **22**, 668.
- POPE, C.H. (1960) Retinal capillary microaneurysms: a theory of pathogenesis. *Diabetes*, **9**, 9.
- RATNOFF, O.D., & MENZIE, C. (1951) Determination of fibrinogen in small samples of plasma. *Journal of Laboratory and Clinical Medicine*, **37**, 316.
- SALINAS-MADRIGAL, L., PIRANI, C.L. & POLLAK, V.E. (1970) Glomerular and vascular 'insudative' lesions of diabetic nephropathy. *American Journal of Pathology*, **59**, 369.
- SCHONEBECK, L., WEBER, V. & VOIGT, K.D. (1962) Protein-bound carbohydrate-containing constituents of serum and vessel wall in atherosclerosis. *Journal of Atherosclerosis Research*, **2**, 332.
- SIPERSTEIN, M.D. (1970) In: *Pathogenesis of Diabetes Mellitus* Nobel Symposium 13, p. 81. (Ed. by E. Cerasi and R. Luft), Almqvist and Wiksell, Stockholm.
- SKANSE, B. (1963) The serum proteins in diabetes mellitus in relation to vascular complications. *Acta Endocrinologica*, **43**, 3.
- SKOVBUURG, F., NIELSON, A.V., SCHLICHTKRULL, J. & DITZEL, J. (1966) Blood viscosity in diabetic patients. *Lancet*, **i**, 129.
- STAMLER, J. (1967) Serum lipids as predictors of atherosclerotic disease. In: *Atherosclerotic Vascular Disease* (Ed. by A. Brest and J. Moyer), Chap. 17, p. 207. Appleton-Century, Crofts, New York.
- STOUT, R. W. (1970) Development of vascular lesions in insulin treated animals fed a normal diet. *British Medical Journal*, **3**, 685.
- STOUT, R. W. & VALLANCE-OWEN, J. (1969) Insulin and atheroma. *Lancet*, **i**, 1078.
- TRAP-JENSEN, J. (1970) Capillary permeability in diabetics. *Clinical Science*, **39**, 39.
- VALDORF-HANSEN, F. (1967) Coagulability in diabetics. *Acta medica scandinavica Suppl.* **476**, 147.

- VAN HANDEL, E. & ZILVERSMIT, D.B. (1957) Micromethod for the direct determination of serum triglycerides. *Journal of Laboratory and Clinical Medicine*, **50**, 152.
- WALKER, F. (1968) The effects of hormones in intercellular ground substance. *Hospital Medicine*, **2**, 589.
- WILSON, D.E., SCHREIBMAN, P.H., DAY, V.C. & ARKY, R.A. (1970) Hyperlipidaemia in an adult diabetic population. *Journal of Chronic Disease*, **23**, 501.
- YDE, H. (1969) Abnormal growth hormone response to ingestion of glucose in juvenile diabetics. *Acta medica scandinavica*, **186**, 499.
- ZEVEERS, A., YARON, E. & GROEN, J.J. (1968) A study of fasting and post-prandial serum triglycerides in connection with epidemiological surveys. *Clinica chimica acta*, **19**, 267.
- ZORRILLA, E., HERNANDEZ, A. & SERRANO, P.A. (1970) Characteristics of hyperlipaemia in diabetes mellitus. *Diabetes Suppl. 1*, **19**, 406.