Review article

Endothelial cell function in diabetic microangiopathy

M. Porta, M. La Selva, P. Molinatti and G. M. Molinatti

Cattedre di Clinica Medica 2 ed Endocrinologia, University of Turin, Turin, Italy

The last 20 years have witnessed a tremendous increase of interest in the role of vascular endothelium in health and disease. The concept of endothelium serving as an inert barrier between blood and the surrounding tissues was first depicted in the middle of the last century. According to that model, molecular exchanges across the vessel wall were simply regulated by the interplay between hydrostatic and osmotic pressures, endothelium serving the sole purpose of providing a physical barrier. Later on, progress in the knowledge of blood clotting and its mechanisms required the addition of a new feature, namely anti-thrombogenicity. It was mainly after the development of methods for culturing endothelial cells [1] that the idea of a passive semi-permeable, anti-coagulating nucleated cellophane gave way to a more complex and dynamic model. We now know that endothelium is a widespread organ of multiple capabilities which is actively involved in a number of heterogeneous processes, including the maintenance of the vessel wall integrity, the blood clotting system, the clearance of circulating lipids, the renin-angiotensin system, the endocrine system, and the immune response.

Endothelium is characteristically damaged in a number of chronic illnesses, notably autoimmune diseases, atherosclerosis and diabetes. This review is aimed at reviewing the current knowledge on those aspects of endothelial pathophysiology that appear more specifically involved in the pathogenesis of diabetic microangiopathy.

Morphology

Endothelium lines the luminal surface of large vessels with a monolayer of polygonal oblong cells stretching their longer diameter along the direction of blood flow. In small vessels this “cobblestone” pattern disappears, as individual cells may fold their cytoplasm to encircle the entire capillary lumen. In both cases, the cells remain firmly attached to the basement membrane and are flattened, producing a high surface/volume ratio.

The intracellular endowment of organelles includes numerous free ribosomes, a well developed rough endoplasmic reticulum and Golgi apparatus, both suggestive of intensive synthetic and metabolic activities, and more specific features such as vesicles, Weibel-Palade bodies [2] and different types of filaments formed by tubulin, vimentin, actin and myosin [3]. Morphological evidence of endothelial alterations in diabetic retinopathy includes both acellular and hypercellular capillaries and microaneurysms [4]. The acellular vessels are not perfused by blood, but it has not been established whether endothelium is necessary to keep the capillaries patent or whether local ischaemia, secondary to capillary occlusion, leads to cell death. In neuropathy, endothelial cell proliferation may obliterate the lumen of sural nerve capillaries [5]. Acellular capillaries have been described in feet muscles of patients with microangiopathy [6] but it was not specified whether they were perfused or not. Intimal proliferation has also been described in medium-sized coronary arteries of diabetic patients [7].

Transmission electron microscopy did not show any specific sign of cell damage in retinal [8, 9], muscle [10], or skin [11] capillaries, although images of doubtful interpretation, such as thinning of glomerular endothelium [12] and a decreased number [13, 14] of enlarged [15] vesicles in the endothelial cells of muscle capillaries and retinal microaneurysms have been reported. No changes were observed in intracellular organelles in the endothelial cells of the retina or the glomeruli [15]. Similarly to observations with tubulin in peripheral nerves [16], non-enzymatic glycosylation might alter some properties of structural proteins, but no specific studies have been carried out on cytoplasmic filaments in diabetes.

Turnover

One study in scanning electron microscopy reported an increased number of argyrophilic elements in the endothelium of poorly-controlled alloxan-diabetic rab-

bits, compared to well-controlled and healthy animals [17]. This finding was interpreted as evidence for accelerated endothelial cell turnover. On the other hand, a number of investigators have shown that, *in vitro*, DNA synthesis is reduced and replication delayed in endothelial cells subjected to supraphysiological concentrations of glucose in the culture medium [18–20]. The above data can be reconciled if one bears in mind that endothelial turnover is a rather slow process in the normal adult vessel wall, where mitotic figures are rarely if ever found. Thymidine labelling indexes are very low in retinal capillaries and in the aorta [21]. Secondly, the repair of de-endothelialised areas appears to involve two mechanisms: migration of nearby elements to fill the gaps, followed by replication to re-establish the original cell density [22]. Experimental evidence suggests that the repair of lesions of the monolayer is not hampered *in vivo* by diabetes [6] nor *in vitro* by high glucose concentrations [20]. Thus, should endothelial cell death increase to some extent in diabetes, exposure of subendothelium underlying the shed elements could still be prevented by spreading of cytoplasm from neighbouring cells, while sufficient replications for local necessities can reasonably be expected to take place in spite of hyperglycaemia. However, it seems unlikely that cells die more rapidly on the wall of patent vessels in diabetes, as this would presumably be preceded by profound morphological alterations which the extensive studies carried out by transmission electron microscopy have failed to show. In his exhaustive investigation of diabetic retinopathy, Ashton [9] could report only one image of a disrupted endothelial cell, with red cells and platelets adhering to the basement membrane. The numerous areas of de-endothelialisation reported by Dolgov [17] in alloxan diabetic rabbits could have been artifactual, as they were not accompanied by platelet adhesion.

Metabolism

The intracellular metabolic pathways of endothelium have not been studied extensively and the few data available have been collected in heterogeneous models, utilising cells from both micro- and macrovessels of different species.

Glucose appears to be metabolised mainly via anaerobic glycolysis, in isolated cells from both large [23] and small vessels [24], a feature that might reflect the ability of endothelial cells to tolerate relatively prolonged periods of hypoxia. *In vitro*, the oxygen consumption decreases when the glucose concentration in the culture medium increases, a phenomenon shared by other tissues, and known as the Crabtree effect [23].

Studies in cultured retinal capillary endothelium provided evidence that these cells have a facilitative diffusion-type transport system for hexoses, and that they always contain free intracellular glucose [25].

Since the latter is likely to increase in the presence of hyperglycaemia, studies have been carried out to establish whether potentiation of the polyol pathway occurs in these cells, leading to sorbitol accumulation and, possibly, osmotic damage. Indeed, increased intracellular amounts of sorbitol have been reported in cultured human umbilical vein endothelial cells [26] and in rabbit aortic intima-media preparations [27] subjected to high glucose. However, aldose reductase, the key enzyme in the polyol pathway, has been localised in the endothelium of rat arteries [28], and rat [29] and human corneas [30] but neither in human [30] nor rat retinal capillaries or rat glomerular capillaries [29]; i.e., the vessels feeding those tissues that are more characteristically involved in diabetic microangiopathy.

Insulin receptors

Specific, high-affinity binding sites for insulin have been demonstrated in endothelial cells of large vessels, such as human umbilical vein and arteries, bovine aorta and pulmonary arteries and veins [31], as well as capillaries of the heart [32], adipose tissue [33], brain [34, 35], retina [36] and liver [37]. These binding sites exhibit the same characteristics of pH and temperature dependence, negative cooperativity and down-regulation as described for insulin receptors of other tissues [31, 38].

One major problem posed by insulin receptors is their role in endothelium. Glucose diffuses rapidly across the cell membrane of these cells [39] and insulin is not required for its uptake [25]. Indeed, physiological concentrations of this hormone stimulate the incorporation of ^{14}C -glucose into glycogen in bovine retinal capillaries but not aortic endothelium, even though the receptor concentration is similar in the two tissues [36]. Other authors have been unable to show any stimulatory effects of insulin on glucose or neutral amino acid metabolism in large vessel endothelial cells [31].

Insulin might be involved in other functions and/or metabolic pathways, as it has been shown to stimulate ^3H -thymidine incorporation in retinal [36], cerebral [40] and adipose [33] microvessels, but again not in large vessel endothelium, such as the aorta [36, 40] or pulmonary artery [33]. This hormone has also been reported to increase the uptake of aminoisobutyric acid in bovine aortic endothelial cells [41] and to increase the number of intracytoplasmic vesicles in striated muscle capillaries [14]. Endothelial uptake might also be involved in the clearance of insulin [31].

The main bulk of the hormone, however, is transferred unaltered from the luminal to the abluminal face of endothelium. This has been elegantly demonstrated *in vitro* [42] by growing a monolayer of calf aorta endothelium on a membrane permeable to insulin, which separated two chambers containing culture medium. When labelled insulin was added to the "lumi-

nal" chamber, transport to the other chamber occurred with a specific, receptor-mediated and saturable mechanism. Internalisation and subsequent release of receptor-bound insulin have also been reported in bovine aortic [38] and brain capillaries [43].

The mechanisms underlying insulin "transcytosis" after binding to the receptors are at present poorly understood. It appears, however, that endothelial cells of both large and small vessels actively transfer insulin from the blood to the tissues and this, in turn, leads to some further considerations.

Firstly, insulin is not as readily available to muscle and adipose tissue as has been thought, since a delaying continuous endothelial monolayer must be crossed before target tissues are reached [39]. In this context, it would be tempting to speculate that the reported increase of insulin resistance in patients with microangiopathy [44] may reflect impaired transendothelial transport of the hormone, i.e. yet another manifestation of widespread endothelial dysfunction associated with the chronic complications of diabetes.

Secondly, the finding of receptors in the capillaries of tissues that, like the brain and retina, are traditionally considered insulin independent poses further problems. Although insulin does not cross the blood-brain barrier [45], its binding to cerebral microvessels might still result in metabolic signals for the underlying brain cells [34]. Insulin is also likely to act as a growth factor for the developing central nervous system and its brain uptake, binding to cerebral microvessels and cerebrospinal fluid brain levels were found to be 8 times as high in newborn rats as in adult animals [46], suggesting a facilitated access for the hormone to the immature central nervous system.

Specific binding sites for insulin-like growth factors (IGF I and IGF II) have been observed in endothelial cells of large vessels (human umbilical vein and artery, bovine aorta, pulmonary artery and vein) [31] and the capillaries of bovine brain [43] and adipose tissue [33]. Similarly to insulin, both growth factors are internalised [43] and stimulate thymidine incorporation into the DNA of small but not large vessel endothelial cells [33]. Insulin does not interact with the receptors for IGF II but exhibits definite cross-reactivity with those for IGF I, suggesting that its stimulatory effect on thymidine incorporation may be mediated through the latter [33].

Capillary permeability

Capillary permeability has progressively decreased during the evolution of species to permit the development of a complex vascular system and, along with it, of larger and more sophisticated organisms [47]. In fact, the survival of complex organisms is based upon an even distribution of small and large molecules throughout the body, which is obtained in higher verte-

brates by increasing the hydrostatic pressure within their vascular system. This, in turn, would lead to massive protein sieving and trapping in the interstitial spaces, which is prevented by the development of a closed and only slightly permeable vascular network and of a lymphatic system to drain large molecules from the interstitium into the venous end of the circulation. However, a "closed" vascular system is never completely sealed, nor would this be desirable. Large protein and even blood cells must be allowed to leave the vessel lumen or, as in the case of hormones, to enter it. Endothelium is the structure controlling these exchanges and has undergone a series of modifications to match local functional requirements in different organs.

In man, at least four histologic types of capillaries are found: sinusoids in the liver, spleen and bone marrow; fenestrated capillaries in the kidney, endocrine glands and gastro-intestinal mucosa; continuous capillaries in striated, cardiac and smooth muscles and, finally, the specialised continuous capillaries of brain and retinal microvessels [48, 49]. In the latter, endothelial cells are joined by continuous zonulae occludentes occasionally reinforced by desmosomes [50], forming the anatomical correlates of the blood-brain barrier and blood-retinal barrier.

Hyperpermeability of the capillary wall to molecules of different size, ranging from small ions [10, 51], to dopamine [52], fluorescein [53–56], albumin [57–59] up to IgG [57] has been reported in patients with microangiopathy. A series of adjustments appears to take place in the diabetic organism in order to avoid protein accumulation in the interstitium and, consequently, an increase of the colloid osmotic pressure. Indeed, a decrease of the latter has been reported, possibly due to overcompensatory capillary filtration of fluid resulting in lymphatic wash-out of interstitial proteins and increased transcapillary colloid osmotic gradient [58]. It is conceivable that this mechanism, while preventing chronic oedema in diabetes, might also account for the above mentioned increased passage of ions and small solutes across the capillary wall.

A number of mechanisms have been suggested to explain the increased transcapillary escape rate of albumin (TER_{Alb}) in microangiopathy. In vitro, glycosylated albumin is avidly taken up by endocytosis in isolated rat capillaries, whereas non-glycosylated albumin is not [60]. Furthermore, in the presence of concentrations of the former mimicking those found in poorly-controlled patients, the latter is ingested, as well. The same authors have also reported that glycosylation of endothelial cell membrane components is able to increase the uptake of glycosylated albumin, albeit not the native protein, into micropinocytotic vesicles. Another set of experiments showed that the intravenous administration of insulin to diabetic patients, in doses that do not produce hypoglycaemia, is accompanied by a decrease of the intravascular pool of albumin and

of plasma volume [61]. When injected into non-treated diabetic rats, insulin was found to acutely normalise the reduced number of intracytoplasmic vesicles in striated muscle capillary endothelium, a finding interpreted as evidence for the re-establishment of transendothelial transport mediated by the hormone [14]. Finally, hypoglycaemia was reported to increase the TER_{Alb} in normal subjects [62] and might occasionally contribute to increased albumin escape from the patients' plasma. However, were these the sole mechanisms at work, one would expect the TER_{Alb} to correlate with metabolic control and/or the degree of hyperinsulinaemia necessary to achieve it. This was not the case in the report of Feldt-Rasmussen et al. [59], showing that the TER_{Alb} was increased in patients with microangiopathy and microalbuminuria, but not in those with normal urinary albumin excretion, and no or minimal retinopathy. The groups had similar age, sex, duration of disease, daily insulin dose, glycated haemoglobin levels and blood pressure.

Thus, abnormal leakage appears to be a manifestation of microangiopathy rather than a mechanism primitively involved in its pathogenesis. Obviously, it is difficult to organise prospective studies to prove such an assumption. Further "cross-sectional" support for this conclusion could be drawn from evidence of increased permeability of the blood-brain barrier to dopamine in patients with microalbuminuria [52] and of the blood-retinal barrier to fluorescein in patients with background retinopathy as compared to patients with clinically unaffected retinas [55]. However, previous data in the literature had reported breakdown of the blood-retinal barrier in the absence of clinically evident retinopathy [53, 54] and the whole issue is complicated by the finding that, of the two components of the blood-retinal barrier, retinal pigment epithelium rather than retinal capillary endothelium may be responsible for fluorescein leakage in early diabetic retinopathy [63]. In any case, capillary hyperpermeability becomes a main feature of later stages of retinopathy, as gross leakage of the dye from most microaneurysms, capillaries and new vessels is characteristically observed on fluorescein angiograms [64].

Increased protein passage across endothelium and deposition on the basement membrane may contribute to the ubiquitous thickening of the latter, which is generally considered the hallmark of diabetic microangiopathy. Albumin and other plasma proteins have been demonstrated in the basement membrane of glomerular [65] and dermal capillaries [66] from diabetic patients, not normal control subjects. In turn, the altered basement membrane may become more permeable to the molecules that reach it, as suggested by the reports of sodium fluorescein passing in increased amounts, and more rapidly, the pericapillary collagen sheath of nail-fold capillaries in diabetes [56]. The diffusion capacity for small ions is also increased in striated muscle, but does not correlate with the basement

membrane thickness in Type 1 (insulin-dependent) diabetes [10].

An indication that imbalanced sorbitol/inositol metabolism may contribute to capillary hyperpermeability can be inferred from the observation that aldose reductase inhibition is able to reduce albumin permeation from new vessels of granulation tissue in streptozotocin diabetic rats [67]. However, in that experiment, newly formed capillaries feeding inflammatory tissue induced by subcutaneous polyester fabric were studied; more representative models of the microcirculation in diabetes would be warranted to substantiate this observation in view of its potential practical implications.

Haemostasis

Recent data have shown that endothelium is actively involved in the synthesis of factors that prevent or limit the progression of blood coagulation as well as others that lead to the activation of platelets and of the plasma clotting system. In other words, the long-recognised non-thrombogenic property of endothelium results from a balance between pro- and anti-coagulant activities, the former prevailing in physiologic conditions.

Circulating platelets do not interact with endothelium but promptly adhere to areas of subendothelium when this is exposed to the blood flow [68]. Following adhesion, platelets may aggregate if further elements are recruited and activated, leading to the formation of a white thrombus [69]. Platelet adhesion to subendothelium requires the presence of von Willebrand factor (vWF) [70], a glycoprotein synthesized by endothelial cells [71] of small and large blood vessels [72], stored in rod-shaped organelles known as Weibel-Palade bodies [73] and deposited by these cells on to the basement membrane [74].

Platelet aggregation can be induced, with separate mechanisms, by at least three mediators: thromboxane A_2 [75], ADP [76] and Platelet Activating Factor (PAF) [77]. In the late 1970s, great emphasis [78] had been placed upon the balance between the platelet-derived thromboxane A_2 and the endothelial synthesized prostacyclin [79], the most potent inhibitor of aggregation and, to an extent, adhesion, known so far [80]. However, further observations have shown that platelet/endothelial interactions are far more complex. Endothelial cells also synthesize and release thromboxane A_2 [81] and PAF [82, 83], thus potentially contributing to activate platelets in certain conditions, whereas the presence of membrane ADPase might play a role in preventing aggregation [84].

A similar co-existence of activating and inhibiting factors has also been observed in the case of the plasma clotting system. On the one hand, heparin-like molecules tightly bound to the endothelial surface [85]

act as co-factors for antithrombin III [86], one of the major physiological anti-coagulant substances, which is itself synthesized by endothelial cells [87]. Another endothelial molecule, thrombomodulin, is involved in the activation of the protein C anticoagulant pathway [88]. On the other hand, thrombin-exposed endothelium synthesizes thromboplastin [89] and exhibits clot retracting activity [90], while endotoxin-treated endothelial cells have been shown to generate tissue factor [91], initiating a pro-coagulant pathway, and to recruit platelets leading to the formation of a fibrin clot [92]. Tissue factor-like pro-coagulant activity is also induced by interleukin I [93].

With regard to the fibrinolytic system, once again endothelial cells have been shown to produce plasminogen activators, including urokinase [94, 95] as well as inhibitors of plasminogen activation [94, 96]. Possibly, activated protein C enhances the activity of the former and reduces that of the latter [97].

Thus, it appears that the role of the vessel wall in the modulation of haemostasis is complex and little understood. In this light, reports of altered production of clotting-related endothelial molecules in diabetic microangiopathy should be considered as merely descriptive, and efforts to fit such data into pathogenetic hypotheses can only be regarded as speculative. Investigations in diabetic subjects have concerned themselves mainly with vWF, prostacyclin and fibrinolysis.

vWF is increased in the plasma of patients with diabetic microangiopathy [98, 99] and its elevation is associated with retinal capillary hyperpermeability to fluorescein [55]. vWF levels correlate with the age of the patients and the duration of diabetes [100], i.e. the major risk factors for the development of microvascular complications [101], but are not influenced by either acute changes of blood sugar or insulin levels [100, 102] or the degree of metabolic control, as assessed by Schlichtkrull's M index [100] and HbA_{1c} [99]. vWF plasma levels may rise in response to stress situations, like hypoglycaemia [103], hyperglycaemic coma [104] and strenuous exercise [105]. In contrast to earlier reports [106], they are not influenced by growth hormone levels [100, 107].

Although vWF is found also in platelets [108] and megakaryocytes [109], its main storage site is endothelium; the question arose whether the latter synthesizes and releases more glycoprotein or becomes unable to keep it intracellularly and lets it leak into the circulation when subjected to conditions of chronic stress in microangiopathy. Studies *in vivo*, in which vWF was measured after inducing its release from vascular endothelium into the plasma by venous stasis [110, 111] or 1-deamino-8-D-arginine vasopressin [112], appear to support the first hypothesis. It is not known whether the clearance of vWF is also affected by diabetic microangiopathy, nor what causes endothelial cells to produce more vWF. A study *in vitro* had reported increased intracellular content of vWF in cultured

endothelium subjected to glucose concentrations mimicking those found in poorly-controlled patients [113].

This glycoprotein appears to be structurally normal in diabetes [111]; and glycosylation, if it occurs, may not alter its function [100, 102]. Finally, its increased levels do not alter platelet function, as assessed *in vitro* by glass bead retention [114] or ADP-induced aggregation [114, 115].

While it is accepted that diabetic platelets have an increased ability to synthesize thromboxane A₂ [116], data on prostacyclin production are contradictory, as the latter has been reported as reduced [117–119], normal [120, 121] and even increased [122, 123]. The perplexities raised by such results may have to remain unanswered for some time to come, as there are still technical problems with the extraction of the stable end-products of prostacyclin from plasma. Work *in vitro* may partially overcome this problem because culture media containing minor amounts of added serum are processed and indicate reduced prostacyclin production by endothelial cells grown in the presence of diabetic serum [124, 125] though not of high glucose concentrations [126].

Antithrombin III activity is decreased in diabetes, due to non-enzymatic glycosylation of its active site, not as a consequence of decreased circulating levels of the molecule [127, 128].

Plasma fibrinolytic activity is reduced in patients with retinopathy both basally and after release of plasminogen activators from endothelium into the blood, as obtained by forearm venous stasis [129]. However, no studies have been carried out *in vivo* or *in vitro* to ascertain if this is due to reduced synthesis of plasminogen activators, increased production of their inhibitors or a combination of both. A further contribution to the impaired fibrinolytic activity of plasma may derive from the increased resistance of non-enzymatically glycosylated fibrin to degradation by plasmin [130].

Angiotensin-converting enzyme

Angiotensin-converting enzyme (ACE) is another circulating glycoprotein synthesized by vascular endothelium [131] which cleaves angiotensin I to angiotensin II and inactivates bradykinin [132]. Two studies have reported supra-normal levels of serum ACE in some diabetic patients. In the first one [133], 18% of 232 control subjects had elevated ACE on a cross-sectional basis and, upon repeating the test several times over 1 year, another 6% showed sporadically increased values. Since the prevalence of retinopathy was higher in the group with high ACE, the authors concluded that this parameter may indicate widespread endothelial damage in microangiopathy. In the second paper [134], 9% of 56 diabetic patients were found with increased serum ACE but vascular complications were not assessed. ACE decreased to normal in 6 patients when

ketoacidosis developed as a result of insulin withdrawal. In our laboratory [135], normal levels of serum ACE were measured in 12 patients with retinopathy and increased plasma vWF. Forearm venous stasis was able to release ACE from endothelium to a similar extent in the diabetic patients and in healthy control subjects.

Taken together, the above results suggest that serum ACE is increased in a minor share of patients with diabetic microangiopathy and may be a poorer indicator of endothelial damage than vWF. Indeed, ACE is more characteristically increased in chronic granulomatous diseases, such as sarcoidosis, Gaucher's disease and leprosy [133].

Angiogenesis

This term indicates the process by which endothelial cells migrate from pre-existing capillaries into avascular sections of various tissues and proliferate to form new vessels. This is a physiological event in embryonic development and in the endometrium, during the menstrual cycle. Pathological new vessels develop in wound healing, inflammatory tissue, tumour growth and diabetic retinopathy.

In the latter condition, neovascularisation occurs in association with large areas of capillary non-perfusion in the peripheral retina. In addition, hypercellular microaneurysms have been interpreted by some authors as abortive attempts at new vessel formation, because they are more often observed at the borders of non-perfused areas [101]. These observations have led to the suggestion that factor(s) released from the ischaemic retina may act as effector(s) of new vessel proliferation but no such factors have been identified with certainty. Elevated concentrations of the angiogenetic fibroblast growth factor were measured in the ocular fluid of diabetic patients undergoing vitrectomy [136]. However, a more complicated interplay may take place among pro- and anti-angiogenetic molecules in the human eye, as inhibitors of endothelial cell proliferation have been reported in the vitreous of non-diabetic as well as diabetic individuals [137]. Furthermore, it may not be necessary to postulate the synthesis of a specific substance to induce endothelial proliferation in diabetic retinopathy. A number of physiologically occurring molecules or their breakdown products are able to induce endothelial cell migration and/or neovascularisation in vivo, including heparin [138], PGE₁ [139], PGE₂ [140], hyaluronic acid degradation products [141], and angiotensin II [142]. Angiogenesis can also be induced in vivo by human follicular fluid [143] and extracts from myocardial infarcts [144] and is facilitated by copper ions, fibronectin and some gangliosides [139].

Conclusions

Endothelial cells are directly exposed to altered concentrations of circulating metabolites in diabetes and

are likely to be involved early, if not primarily, in the natural history of the chronic complications of the disease. Thus, better understanding of the mechanisms underlying endothelial dysfunction and of their possible pathogenetic relevance might help to establish a rationale on which to base prospective trials for the prevention or treatment of microangiopathy.

A word of caution should be made in warning that most data available today are derived from experiments in vitro or carried out under highly perturbed conditions in vivo and that virtually none of the methods employed has so far been standardised among different laboratories. Hence, it should come as no surprise if some results are in conflict with each other or will be refuted in the near future. Work on endothelium has only just begun and much still has to be done in this fascinating field of investigation.

Acknowledgements. This paper was supported by the Consiglio Nazionale delle Ricerche, Progetto Finalizzato "Medicina Preventiva", Sottoprogetto "Malattie Degenerative", Grant No. 86.01866.56. MLS is recipient of a grant from Regione Piemonte, Progetto Finalizzato 6.

References

1. Jaffe EA, Nachman RL, Becker CG, Minick CR (1973) Culture of human endothelial cells derived from umbilical veins: identification by morphologic and immunologic criteria. *J Clin Invest* 52: 2745-2756
2. Weibel ER, Palade GE (1964) New cytoplasmic components in arterial endothelia. *J Cell Biol* 23: 101-112
3. Drenkhahn D (1983) Cell motility and cytoplasmic filaments in vascular endothelium. *Prog Appl Microcirc* 1: 53-70
4. Kohner EM, Henkind P (1970) Correlation of fluorescein angiogram and retinal digest in diabetic retinopathy. *Am J Ophthalmol* 69: 403-414
5. Dyck PJ, Hansen S, Karnes J, O'Brien P, Yasuda H, Winkbank A, Zimmermann B (1985) Capillary number and percentage closed in human diabetic sural nerve. *Proc Natl Acad Sci USA* 82: 2513-2517
6. Tilton RG, Faller AM, Burkhard JK, Hoffmann PL, Kilo C, Williamson JR (1985) Pericyte degeneration and acellular capillaries are increased in the feet of human diabetic patients. *Diabetologia* 28: 895-900
7. Blumental HT, Alex M, Goidenberg S (1960) A study of lesions of the intramural coronary artery branches in diabetes mellitus. *Arch Pathol* 70: 27-42
8. Bloodworth JMB (1963) Diabetic microangiopathy. *Diabetes* 12: 99-114
9. Ashton N (1974) Vascular basement membrane changes in diabetic retinopathy. *Br J Ophthalmol* 58: 344-366
10. Leinonen H, Matikainen E, Juntunen J (1982) Permeability and morphology of skeletal muscle capillaries in Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 22: 158-162
11. Aagenaes O, Moe H (1961) Light and electron microscope study of skin capillaries of diabetics. *Diabetes* 10: 253-259
12. Bloodworth JMB (1978) A re-evaluation of diabetic glomerulosclerosis 50 years after the discovery of insulin. *Hum Pathol* 9: 439-453
13. Ravid M, Silman-Socher R, Ben Shaul Y, Sohar E (1976) Quantitative electron microscopic study of capillaries in diabetes mellitus. *Beitr Path Bd* 159: 280-291
14. Østerby R, Gundersen HJG, Christensen NJ (1978) The acute effect of insulin on capillary endothelial cells. *Diabetes* 27: 745-749

15. Bloodworth JMB, Molitor DL (1965) Ultrastructural aspects of human and canine diabetic retinopathy. *Invest Ophthalmol* 4: 1037-1048
16. Williams SH, Howarth NL, Devenny JJ, Bitensky MW (1982) Structural and functional consequences of increased tubulin glycosylation in diabetes mellitus. *Proc Natl Acad Sci USA* 79: 6546-6550
17. Dolgov VV, Zaukina OE, Bondarenko MF, Repin US (1982) Aortic endothelium of alloxan diabetic rabbits: a quantitative study using scanning electron microscopy. *Diabetologia* 22: 338-343
18. Stout RW (1982) Glucose inhibits replication of cultured human endothelial cells. *Diabetologia* 23: 436-439
19. Lorenzi M, Cagliero E, Toledo S (1985) Glucose toxicity for human endothelial cells in culture. Delayed replication, disturbed cell cycle, and accelerated death. *Diabetes* 34: 621-627
20. Porta M, La Selva M, Bertagna A, Molinatti GM (1986) Glucose in high concentrations inhibits DNA synthesis and replication, but not migration, of cultured human endothelial cells by osmotic and non osmotic mechanisms. *Diabetologia* 29: 583-584 (Abstract)
21. Payling Wright H (1970) Endothelial turnover. In: Brinkhous KM, Koller F, Biggs R, Rodman NF, Hinnom S (eds) *Vascular factors and thrombosis*. Schattauer, Stuttgart, pp 79-87
22. Sholley MM, Gimbrone MA, Cotran RS (1977) Cellular migration and replication in endothelial regeneration. *Lab Invest* 36: 18-25
23. Dobrina A, Rossi F (1983) Metabolic properties of freshly isolated bovine endothelial cells. *Biochim Biophys Acta* 762: 295-301
24. McCall AL, Gould JB, Ruderman NB (1984) Diabetes-induced alterations of glucose metabolism in rat cerebral microvessels. *Am J Physiol* 247: E462-E467
25. Betz AL, Bowman PD, Goldstein GW (1983) Hexose transport in microvascular endothelial cells cultured from bovine retina. *Exp Eye Res* 36: 269-277
26. Koh MS, Misch KJ, Yuen CT, Rhodes EL (1986) Accumulation of sorbitol in endothelial cells. A possible cause of diabetic microangiopathy. *Diabetes Res* 3: 217-219
27. Morrison AD, Clements RS, Winegrad AI (1972) Effects of elevated glucose concentrations on the metabolism of the aortic wall. *J Clin Invest* 51: 3114-3123
28. Ludwigson MA, Sorenson RL (1980) Immunohistochemical localization of aldose reductase. I. Enzyme purification and antibody preparation. Localization in peripheral nerve, artery and testis. *Diabetes* 29: 438-449
29. Ludwigson MA, Sorenson RL (1980) Immunohistochemical localization of aldose reductase. II. Rat eye and kidney. *Diabetes* 29: 450-459
30. Akagi Y, Yajima Y, Kador PF, Kuwabara T, Kinoshita JH (1984) Localization of aldose reductase in the human eye. *Diabetes* 33: 562-566
31. Bar RS (1982) Interaction of insulin and insulin-like growth factors (IGF) with endothelial cells. *Ann NY Acad Sci* 401: 150-162
32. Bar RS, De Rose A, Sandra A, Peacock ML, Owen WG (1983) Insulin binding to microvascular endothelium of intact heart: a kinetic and morphometric analysis. *Am J Physiol* 244: E447-E452
33. Bar SR, Dolash S, Dake BL, Boes M (1986) Cultured capillary endothelial cells from bovine adipose tissue: a model for insulin binding and action in microvascular endothelium. *Metabolism* 35: 317-322
34. Frank HJL, Pardridge WM (1981) A direct in vitro demonstration of insulin binding to isolated brain microvessels. *Diabetes* 30: 757-761
35. Van Houten M, Posner BI (1979) Insulin binds to brain blood vessels in vivo. *Nature* 282: 623-625
36. King GL, Buzney SM, Kahn CR, Hetu N, Buchwald S, Macdonald SG, Rand LI (1983) Differential responsiveness to insulin of endothelial and support cells from micro and macrovessels. *J Clin Invest* 71: 974-979
37. Soda R, Tavassoli M (1983) Distribution of insulin receptors in liver cell suspensions using a minibead probe. Highest density is on endothelial cell. *Exp Cell Res* 145: 389-395
38. Jialal I, King GL, Buchwald S, Kahn CR, Crettaz M (1984) Processing of insulin by bovine endothelial cells in culture: internalization without degradation. *Diabetes* 33: 794-800
39. Rasio E (1982) The capillary barrier to circulating insulin. *Diabetes Care* 5: 158-161
40. Vinters HV, Berliner JA, Beck DW, Maxwell K, Bready JV, Cancilla PA (1985) Insulin stimulates DNA synthesis in cerebral microvessel endothelium and smooth muscle. *Diabetes* 34: 964-969
41. Berliner JA, Frank HJL, Karasic D, Capdeville M (1984) Lipoprotein-induced insulin resistance in aortic endothelium. *Diabetes* 33: 1039-1044
42. King GL, Johnson SM (1985) Receptor-mediated transport of insulin across endothelial cells. *Science* 227: 1583-1586
43. Frank HJL, Pardridge WH, Morris WL, Rosenfeld RG, Choi TB (1986) Binding and internalization of insulin and insulin-like growth factors by isolated brain microvessels. *Diabetes* 35: 654-661
44. Maneschi F, Mashiter K, Kohner EM (1983) Insulin resistance and insulin deficiency in diabetic retinopathy of non-insulin-dependent diabetes. *Diabetes* 32: 82-87
45. Goodner JC, Berrie MA (1977) The failure of rat hypothalamic tissues to take up labeled insulin in vivo or to respond to insulin in vitro. *Endocrinology* 101: 605-612
46. Frank HJL, Jankovic-Vokes T, Pardridge WM, Morris WL (1985) Enhanced insulin binding to blood-brain barrier in vivo and to brain microvessels in vitro in newborn rabbits. *Diabetes* 34: 728-733
47. Casley-Smith JR (1980) Comparative fine structure of the microvasculature and endothelium. In: Altura BM (ed) *Vascular endothelium and basement membranes*. Karger, Basel, pp 1-44
48. Fawcett DW (1963) Comparative observations on the fine structure of blood capillaries. In: Orbison JL, Smith DE (eds) *The peripheral blood vessels*. Williams and Wilkins, Baltimore, pp 17-44
49. Bloom W, Fawcett DW (1968) *A textbook of histology*. Saunders, Philadelphia
50. Shakib M, Cunha-Vaz JG (1966) Studies on the permeability of the blood-retinal barrier. 4. Junctional complexes of the retinal vessels and their role in the permeability of the blood-retinal barrier. *Exp Eye Res* 5: 229-234
51. Trap-Jensen J, Lassen NA (1968) Increased capillary diffusion capacity for small ions in skeletal muscle in long-term diabetics. *Scand J Clin Lab Invest* 21: 116-122
52. Lorenzi M, Karam JH, McIlroy MB, Forsham PH (1980) Increased growth hormone response to dopamine infusion in insulin dependent diabetic subjects. *J Clin Invest* 65: 146-153
53. Cunha-Vaz JG, Fonseca JR, Abreu JF, Ruas MA (1979) Detection of early retinal changes in diabetes by vitreous fluorophotometry. *Diabetes* 28: 16-19
54. Waltman SR, Oestrick C, Krupin T, Hamish S, Ratzan S, Santiago J, Kilo C (1978) Quantitative vitreous fluorophotometry: a sensitive technique for measuring early breakdown of the blood-retinal barrier in young diabetic patients. *Diabetes* 27: 85-87
55. Porta M, Townsend C, Clover GM, Nanson M, Alderson AR, McCraw A, Kohner EM (1981) Evidence for functional endothelial cell damage in early diabetic retinopathy. *Diabetologia* 20: 597-601
56. Bollinger A, Frey J, Jäger K, Furrer J, Seglias J, Siegenthaler W (1982) Patterns of diffusion through skin capillaries in patients with long-term diabetes. *N Engl J Med* 307: 1305-1310
57. Parving H-H (1976) Increased microvascular permeability to plasma proteins in short- and long-term juvenile diabetics. *Diabetes* 25 (Suppl 2): 884-889
58. Fauchald P, Norseth J, Jervell J (1985) Transcapillary colloid osmotic gradient, plasma volume and interstitial fluid volume in long-term Type 1 (insulin-dependent) diabetes. *Diabetologia* 28: 269-273

59. Feldt-Rasmussen B (1986) Increased transcapillary escape rate of albumin in Type 1 (insulin-dependent) diabetic patients with microalbuminuria. *Diabetologia* 29: 282-286
60. Williams SK, Devenny JJ, Bitensky MW (1981) Micropinocytic ingestion of glycosylated albumin by isolated microvessels: possible role in pathogenesis of diabetic microangiopathy. *Proc Natl Acad Sci USA* 78: 2393-2397
61. Gundersen HJG, Christensen NJ (1977) Intravenous insulin causing loss of intravascular water and albumin and increased adrenergic nervous activity in diabetics. *Diabetes* 26: 551-557
62. Hilsted J, Petersen F, Madsbad S, Parving H-H, Christensen NJ, Adelhøj B, Bigler D, Sjøtoft E (1985) Changes in plasma volume, in transcapillary escape rate of albumin and in subcutaneous blood flow during hypoglycaemia in man. *Clin Sci* 69: 273-277
63. Tso MOM, Cunha-Vaz JK, Shih C-Y, Jones CW (1980) Clinicopathologic study of blood-retinal barrier in experimental diabetes mellitus. *Arch Ophthalmol* 98: 2032-2040
64. Kohner EM, Porta M (1980) Vascular abnormalities in diabetes and their treatment. *Trans Ophthalmol Soc UK* 100: 440-444
65. Westberg NG (1976) Biochemical alterations of the human glomerular basement membrane in diabetes. *Diabetes* 25 (Suppl 2): 920-924
66. Chavers B, Etzwiler D, Michael AF (1981) Albumin deposition in dermal capillary basement membrane in insulin-dependent diabetes mellitus. A preliminary report. *Diabetes* 30: 275-278
67. Williamson JR, Chaug K, Rowold E, Marvel J, Tomlinson M, Sherman WR, Ackermann KE, Kilo C (1985) Sorbinil prevents diabetes-induced increases in vascular permeability but does not alter collagen cross-linking. *Diabetes* 34: 703-705
68. Baumgartner HR (1972) Platelet interactions with vascular structures. *Thromb Diath Haemorrh* 51 (Suppl): 161-176
69. Honour AJ, Pickering GW, Sheppard BL (1971) Ultrastructure and behaviour of platelet thrombi in injured arteries. *Br J Exp Pathol* 52: 482-494
70. Tschopp BT, Weiss HJ, Baumgartner HR (1974) Decreased adhesion of platelets to subendothelium in von Willebrand's disease. *J Lab Med* 83: 296-300
71. Jaffe EA, Hoyer LW, Nachman RL (1974) Synthesis of von Willebrand factor by cultured human endothelial cells. *Proc Natl Acad Sci* 71: 1906-1909
72. Zetter BR (1981) The endothelial cells of large and small blood vessels. *Diabetes* 30 (Suppl 2): 24-28
73. Wagner DD, Olmsted JB, Marder VJ (1982) Immunolocalization of von Willebrand protein in Weibel-Palade bodies of human endothelial cells. *J Cell Biol* 95: 355-360
74. Sussman II, Rand JH (1982) Subendothelial deposition of von Willebrand's factor requires the presence of endothelial cells. *J Lab Clin Med* 100: 526-532
75. Bhagwat SS, Hamann PR, Still WC, Bunting S, Fitzpatrick FA (1985) Synthesis and structure of the platelet aggregation factor thromboxane A₂. *Nature* 315: 511-513
76. Born GVR (1962) Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 194: 927-929
77. Camussi G, Arese P, Tetta C, Bussolino F, Coda R, Vercellone A (1983) Platelet activating factor. In: Bertani T, Remuzzi G (eds) *Glomerular injury 300 years after Morgagni*. Wichtig, Milan, pp 89-118
78. Moncada S, Vane JR (1979) Arachidonic acid metabolites and the interaction between platelets and blood-vessel walls. *N Engl J Med* 300: 1142-1147
79. Moncada S, Gryglewski R, Bunting S, Vane JR (1976) An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature* 263: 663-665
80. Weiss HJ, Turitto VT (1979) Prostacyclin (Prostaglandin I₂, PGI₂) inhibits platelet adhesion and thrombus formation on subendothelium. *Blood* 53: 244-250
81. Ingerman-Wojenski C, Silver MJ, Smith JB, Macarak E (1981) Bovine endothelial cells in culture produce thromboxane as well as prostacyclin. *J Clin Invest* 67: 1292-1296
82. Camussi G, Pawlowski I, Bussolino F, Caldwell PRB, Brentjens J, Andres G (1983) Release of platelet activating factor in rabbits with antibody-mediated injury of the lung: the role of leukocytes and of pulmonary endothelial cells. *J Immunol* 131: 1802-1807
83. Prescott SM, Zimmerman GA, McIntyre TM (1984) Human endothelial cells in culture produce platelet activating factor (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine) when stimulated with thrombin. *Proc Natl Acad Sci USA* 81: 3534-3538
84. Lieberman GE, Leake DS, Peters TJ (1982) Subcellular localization of adenosine diphosphatase in cultured pig arterial endothelial cells. *Thromb Haemost* 47: 249-253
85. Marcum JA, McKenney JB, Rosemberg RD (1984) Acceleration of thrombin-antithrombin complex formation in rat hindquarters via heparinlike molecules bond to the endothelium. *J Clin Invest* 74: 341-350
86. Busch C, Owen WG (1982) Identification in vitro of an endothelial cell surface cofactor for antithrombin III. Parallel studies with isolated perfused rat hearts and microcarrier cultures of bovine endothelium. *J Clin Invest* 69: 726-729
87. Chan TK, Chan V (1981) Antithrombin III, the major modulator of intravascular coagulation, is synthesized by human endothelial cells. *Thromb Haemost* 46: 504-506
88. Esmon CT, Owen WG (1981) Identification of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. *Proc Natl Acad Sci USA* 78: 2249-2252
89. Brox JH, Østerud B, Bjørklid E, Fenton JW (1984) Production and availability of thromboplastin in endothelial cells: the effects of thrombin, endotoxin and platelets. *Br J Haematol* 57: 239-246
90. Barbieri G, Balconi G, Dejana E, Donati MB (1981) Evidence that vascular endothelial cells can induce the retraction of fibrin clots. *Proc Soc Exp Biol Med* 168: 204-207
91. Colucci M, Balconi G, Lorenzet R, Pietra A, Locati D, Donati MB, Semeraro N (1983) Cultured human endothelial cells generate tissue factor in response to endotoxin. *J Clin Invest* 71: 1893-1896
92. Stern D, Nawroth P, Handley D, Kisiel W (1985) An endothelial cell-dependent pathway of coagulation. *Proc Natl Acad Sci USA* 82: 2523-2527
93. Bevilacqua MP, Pober JS, Majeau GR, Cotran RS, Gimbrone MA (1984) Interleukin 1 (IL 1) induces biosynthesis and cell surface expression of procoagulant activity in human vascular endothelial cells. *J Exp Med* 160: 618-623
94. Loskutoff DJ, Edgington TS (1977) Synthesis of a fibrinolytic activator and inhibitor by endothelial cells. *Proc Natl Acad Sci USA* 74: 3903-3907
95. Levin EG, Loskutoff DJ (1982) Cultured bovine endothelial cells produce both urokinase and tissue-type plasminogen activators. *J Cell Biol* 94: 631-636
96. Esnard F, Dupuy E, Dosne AM, Bodevin E (1982) Partial characterization of a fibrinolytic inhibitor produced by cultured endothelial cells derived from human umbilical vein. *Thromb Haemostas* 47: 128-131
97. Sakata Y, Curriden S, Lawrence D, Griffin JH, Loskutoff DJ (1985) Activated protein C stimulates the fibrinolytic activity of cultured endothelial cells and decreases antiactivator activity. *Proc Natl Acad Sci USA* 82: 1121-1125
98. Bensoussan D, Levy-Toledano S, Passa P, Caen J, Canivet J (1975) Platelet hyperaggregation and increased plasma level of von Willebrand factor in diabetic patients with retinopathy. *Diabetologia* 11: 307-312
99. Collier BS, Frank RN, Milton RC, Gralnick HR (1978) Plasma cofactors of platelet function: correlation with diabetic retinopathy and haemoglobin. A_{1a-c}. Studies in diabetic patients and normal persons. *Ann Int Med* 88: 311-316
100. Porta M, Maneschi F, White MC, Kohner EM (1981) Twenty-four hour variations of von Willebrand factor and factor VIII-related antigen in diabetic retinopathy. *Metabolism* 30: 695-699
101. Kohner EM, McLeod D, Marshall J (1982) Diabetic eye disease. In: Keen H, Jarret J (eds) *Complications of diabetes*, 2nd edn. Arnold, London, pp 19-108
102. Porta M, Kohner EM, Molinatti GM (1987) In vivo studies of

- endothelial cell function in diabetic microangiopathy. In: Belfiore F, Molinatti GM, Williamson JR (eds) *Frontiers in diabetes*, Vol 8. Vascular and neurologic complication of diabetes mellitus. Karger, Basel, pp 16–28
103. Bloom AL (1979) The biosynthesis of factor VIII. *Clin Haematol* 8: 53–77
 104. Paton RC (1981) Haemostatic changes in diabetic coma. *Diabetologia* 21: 172–177
 105. Trovati M, Tamponi G, Marra S, Lorenzati R, Schinco P, Bazzan M, Vitali S, Cavalot F, Pagano G, Lenti G (1983) Exercise-induced changes of factor VIII complex in healthy subjects and in type-I diabetics: relation between growth hormone and von Willebrand factor increments. *Horm Metabol Res* 15: 316–320
 106. Sarji KE, Levine JH, Nair RMG, Sagel J, Colwell JA (1977) Relation between growth hormone levels and von Willebrand factor activity. *J Clin Endocrinol Metab* 45: 853–856
 107. Gonzalez J, Colwell JA, Sarji KE, Nair RMG, Sagel J (1980) Effect of metabolic control with insulin on plasma von Willebrand factor activity (VIII R:WF) in diabetes mellitus. *Thromb Res* 17: 261–266
 108. Howard MA, Montgomery DC, Hardisty RM (1974) Factor-VIII-related antigen in platelets. *Thromb Res* 4: 617–624
 109. Nachman R, Levine R, Jaffe EA (1977) Synthesis of factor VIII antigen by cultured guinea pig megakaryocytes. *J Clin Invest* 60: 914–921
 110. Giustolisi R, Musso R, Russo M, Catania N, Lombardo T, Cacciola E (1982) Possible evidence for an increased factor VIII antigen synthesis in vascular endothelium of diabetic subjects. *Thromb Haemost* 47: 293
 111. Porta M, Ricchetti I, La Selva M, Bertagna A, Molinatti GM (1984) Quantitative and qualitative assessment of plasma von Willebrand factor variations, as induced by forearm venous stasis in patients with diabetic microangiopathy. *Diabetes Res* 1: 219–221
 112. Porta M (1982) Availability of endothelial von Willebrand factor and platelet function in diabetic patients infused with a vasopressin analogue. *Diabetologia* 23: 452–455
 113. Mordes DB, Lazarchick J, Colwell JA, Sens DA (1983) Elevated glucose concentrations increase factor VIII R:Ag levels in human umbilical vein endothelial cells. *Diabetes* 32: 876–878
 114. Porta M, Cagliero E, Kohner EM (1982) Is the pro-adhesive activity of plasma von Willebrand factor counteracted by a physiological inhibitor of platelet adhesiveness? *Clin Sci* 62: 239–242
 115. Porta M, McCraw A, Kohner EM (1982) Inverse relationship between ristocetin co-factor levels and platelet aggregation in insulin-dependent diabetes. *Thromb Res* 25: 507–512
 116. Mustard JF, Packam MA (1984) Platelets and diabetes mellitus. *N Engl J Med* 311: 665–667
 117. Johnson M, Harrison HE, Raftery AT, Elder JB (1979) Vascular prostacyclin may be reduced in diabetes in man. *Lancet* 1: 325–326
 118. Silberbauer K, Scherthaner G, Sinzinger H, Piza-Katzer H, Winter M (1979) Decreased prostacyclin in juvenile onset diabetics. *N Engl J Med* 300: 366–367 (Letter)
 119. Webster J, Lewis PJ, MacDermot, Hensby CN, Kohner EM, Porta M (1981) Forearm ischaemia as a test of prostacyclin production: studies in normal subjects and in patients with diabetes mellitus. *Prostaglandins Med* 6: 661–667
 120. Davis TME, Bown E, Finch DR, Mitchell MD, Turner RC (1981) In vitro venous prostacyclin production, plasma 6-Keto-prostaglandin $F_{1\alpha}$ concentrations, and diabetic retinopathy. *Br Med J* 282: 1259–1262
 121. Brunner D, Klinger J, Weisbort J, Tuval M, Nakash J, Rosenberg CH, Nissim S (1984) Thromboxane, prostacyclin, beta-thromboglobulin, and diabetes mellitus. *Clin Ther* 6: 636–642
 122. Ylikorkala O, Kaila J, Viinikka L (1981) Prostacyclin and thromboxane in diabetes. *Br Med J* 283: 1148–1150
 123. Lane LS, Jansen PD, Lahav M, Rudy C (1982) Circulating prostacyclin and thromboxane levels in patients with diabetic retinopathy. *Ophthalmology* 89: 763–766
 124. Paton RC, Guillot R, Passa P, Canivet J (1981) Prostacyclin production by human endothelial cells cultured in diabetic serum. *Diabetologia* 21: 313–314
 125. Patel MKN, Evans CE, McEvoy FA (1983) 6-keto prostaglandin $F_{1\alpha}$ production in endothelial cell cultures in response to normal and diabetic human serum. *Biosci Rep* 3: 53–60
 126. Weimann BJ, Lorch E, Baumgartner HR (1984) High glucose concentrations do not influence replication and prostacyclin release of human endothelial cells. *Diabetologia* 27: 62–63
 127. Ceriello A, Dello Russo P, Zuccotti C, Florio A, Nazzaro S, Pietrantuono C, Rosato GB (1983) Decreased antithrombin III activity in diabetes may be due to non-enzymatic glycosylation. A preliminary report. *Thromb Haemostas* 50: 633–634
 128. Brownlee M, Vlassara H, Cerami A (1984) Inhibition of heparin-catalysed human antithrombin III activity by non-enzymatic glycosylation: possible role in fibrin deposition in diabetes. *Diabetes* 33: 532–535
 129. Almèr LO, Pandolfi M (1976) Fibrinolysis and diabetic retinopathy. *Diabetes* 25 (Suppl 2): 807–810
 130. Brownlee M, Vlassara H, Cerami A (1983) Nonenzymatic glycosylation reduces the susceptibility of fibrin to degradation by plasmin. *Diabetes* 32: 680–684
 131. Caldwell PRB, Seegal BC, Hsu KC, Das M, Soffer RL (1976) Angiotensin-converting enzyme: vascular endothelial localization. *Science* 191: 1050–1051
 132. Dorer FE, Kahn JR, Lentz KE, Levine M, Skeggs LT (1974) Hydrolysis of bradykinin by angiotensin-converting enzyme. *Circ Res* 34: 824–827
 133. Lieberman J, Sastre A (1980) Serum angiotensin-converting enzyme: elevations in diabetes mellitus. *Ann Intern Med* 93: 825–826
 134. Schmitz O, Römer FK, Alberti KGMM, Hreidarsson AB, Ørskov H (1985) Angiotensin-converting enzyme in diabetes mellitus. Dependence on metabolic aberration. *Diabetes Metab* 9: 179–182
 135. Porta M, Passera P, Bertagna A, La Selva M, Ricchetti I, Molinatti GM (1987) Levels of serum angiotensin-converting enzyme before and after forearm venous stasis in diabetic microangiopathy. *Diabetes Res* 4: 117–120
 136. Baird A, Culler F, Jones KL, Guillemin R (1985) Angiogenic factor in human ocular fluid. *Lancet* 2: 563 (Letter)
 137. Jacobson B, Sullivan D, Raymond L, Basu PK, Hasany SM (1983) Further studies on a vitreous inhibitor of endothelial cell proliferation. *Exp Eye Res* 36: 447–449
 138. Azizkhan RG, Azizkhan JC, Zetter BR, Folkman J (1980) Mast cell heparin stimulates migration of capillary endothelial cells in vitro. *J Exp Med* 152: 931–944
 139. Gullino PM (1986) Considerations on the mechanism of the angiogenic response. *Anticancer Research* 6: 153–158
 140. Form DM, Auerbach R (1983) PGE_2 and angiogenesis. *Proc Soc Exp Biol Med* 172: 214–218
 141. West DC, Hampson IN, Arnold F, Kumar S (1985) Angiogenesis induced by degradation products of hyaluronic acid. *Science* 228: 1324–1326
 142. Fernandez LA, Twickler J, Mead A (1985) Neovascularization produced by angiotensin II. *J Lab Clin Med* 105: 141–145
 143. Frederick JL, Shimanuki T, di Zerega GS (1984) Initiation of angiogenesis by human follicular fluid. *Science* 224: 389–390
 144. Kumar S, West D, Shahabuddin S, Arnold F, Haboubi N, Reid H, Carr T (1983) Angiogenesis factor from human myocardial infarcts. *Lancet* 2: 364–368

Dr. Massimo Porta
Cattedra di Endocrinologia
Università di Torino
Corso Polonia 14
I-10126 Torino
Italy